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TRANSACTIONS

OF THE

ROYAL SOCIETY OF SOUTH AFRICA

VOLUME XXXVI—PART I

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TRANSACTIONS

OF THE

ROYAL SOCIETY OF SOUTH AFRICA

VOLUME XXXVI

OBITUARIES

JOHN JACKSON

Dr. John Jackson, C.B.E., F.R.S., a former President of the Society, died in Epsom Hospital on December 9, 1958. He was born in Scotland on February 11, 1887, and after a brilliant career at school went up to Glasgow University with the intention of becoming a chemist. Instead he came under the influence of the Professor of Astronomy, Ludwig Becker, and became an astronomer. Jackson migrated to Trinity College, Cambridge, in 1909 and, taking the Mathematical Tripos in 1912, obtained a First Class and special distinctions in Spherical Astronomy and Celestial Mechanics. For this he was awarded the Tyson Medal and a Sheepshanks Exhibition. There followed a further two years at Cambridge during which Jackson worked on the problem of retrograde satellite orbits with particular reference to the recently discovered eighth satellite of Jupiter. In 1914 he won a Smith's Prize and left Cambridge to succeed Sydney Chapman as a Chief Assistant at the Royal Observatory, Greenwich. There he remained, with the exception of a war-time interlude as an officer in the Survey Section of the Royal Engineers, until 1933 when he succeeded Sir Harold Spencer Jones as H.M. Astronomer at the Cape. On his retirement in 1950 Jackson returned to Britain for what he originally intended to be a relatively short holiday but he found life there congenial and bought a house at Ewell which is within easy reach of London and of the various societies whose meetings he wished to attend.

At both Greenwich and the Cape, Jackson threw himself so completely into the work of the observatory as a whole that much of his work was done in collaboration with others and is buried in the semi-anonymity of observatory annals. His first important assignment at Greenwich was to collect and work up the observations of binary stars that had been made there with the 28-inch refractor over a period of many years. His second was to discuss the performance of the Shortt free-pendulum clocks that had been introduced in 1924. He detected the non-uniformity of sidereal time due to the nutation of the earth's axis, a result which led directly to the introduction of 'mean sidereal time' as distinct from 'apparent sidereal time'. He sought evidence for the variability in the rate of rotation of the earth but concluded that the Shortt clocks, unlike the quartz crystal clocks that replaced them, were just not good enough to show up such variations.

Another important piece of work he carried out while at Greenwich was the careful reduction, in collaboration with Dr. H. Knox-Shaw, of the observations of the sun, moon, planets and stars which had been made at the Radcliffe Observatory, Oxford, by Hornsby during the years 1774 to 1798. These relatively accurate observations had not previously been reduced and were particularly important in connexion with the determination of the proper motion of the stars and the investigation of the orbits of the sun, moon and planets.

At the Cape, Jackson took some part in nearly all the work of the observatory but his chief interest was in the parallax programme. This programme, begun in 1926 by Sir Harold Spencer Jones, sought to determine the distances of the nearer southern stars by direct trigonometrical measurements. Jackson threw himself into the long and tedious work involved with his customary enthusiasm and, during the war period, carried on the work almost unaided. By the time of his retirement the programme was virtually complete and the distances of over 1600 stars had been found while the observational material for finding the distances of 200 more had been amassed.

Among Jackson's other astronomical interests was the observation of total eclipses of the sun. He took part in five expeditions: to Giggleswick in Yorkshire in 1927, to Alor Star, Kedah, Malaya, in 1929, to Parent, Canada, in 1932, to Calvinia in 1940, and to Strömstad, Sweden, in 1954. Only the 1929 expedition was completely spoilt by cloud. The 1954 expedition was a joint effort by the Royal Astronomical Society and the British Astronomical Association and was meant more for pleasure than for serious observation.

Jackson's abilities were well known to his fellow astronomers and he took a prominent part in their gatherings. He was Secretary of the Royal Astronomical Society of London from 1924 to 1929 and its President from 1953 to 1955, President of the Commission on Meridian Astronomy of the International Astronomical Union from 1935 to 1938 and of the Commission on Stellar Parallaxes from 1938 to 1952, and President of the Astronomical Society of South Africa in 1935 and 1947. He was elected a Fellow of the Royal Society of London in 1938 and in 1952 was awarded the much-coveted Gold Medal of the Royal Astronomical Society. His long and faithful service to the Royal Observatories was fittingly recognized in the 1950 Birthday Honours when he was created a C.B.E.

Jackson was an extremely hard and conscientious worker. He enjoyed routine observing but was probably happiest when computing. He was a man of simple tastes and sincere religious convictions, very approachable, quite free from any trace of pretension and exactly the same in manner whether he was talking to the Governor-General or to an African workman. He could not abide humbug and had no patience with astrologers or astrology. Of a sociable disposition, he enjoyed meetings of all sorts and was a faithful and hard-working member of several societies in all of which he was well loved and much respected.

He joined the Royal Society of South Africa in 1933, was elected a Fellow in 1934 and served on the Council for many years. He was President of the Society for the year 1949–50.

R. H. S.

ASTLEY JOHN HILARY GOODWIN

By the death of the President, Professor Astley John Hilary Goodwin, on December 5, 1959, the Society, the University of Cape Town, the South African scientific community, and the science of Archaeology suffered an irreparable loss. The loss to the Society was a personal one, for he served it long and zealously. A Member since 1927, he was elected to Fellowship in 1930, to Council in 1932, and to the office of Honorary General Secretary in 1934, a post he filled through seventeen eventful years and relinquished only to give fuller attention to other selfless enterprises, notably the promotion of the South African Archaeological Society which he founded and the Athenaeum Trust which he launched jointly with the late Alexander Logie du Toit to create an appropriate centre for the offices and meetings of the cultural and scientific societies of the Cape Town area. But the interests of the Society remained close to his heart—none were so alert as he to foster its objects, none so fully conversant with its history—and his shrewd advice and genial humour continued to guide many of the decisions of Council. In November 1958 with characteristic modesty he would have declined nomination as President for 1959–60 had the Council been less insistent that, before his failing health should intervene, the Society should confer its highest office upon one who, by distinction in his own field and outstanding service to the Society, merited it so fully.

Professor Goodwin was born at Pietermaritzburg on December 27, 1900, the son of Canon W. A. Goodwin. After attending St. Johns College, Johannesburg, he graduated in Archaeology at Cambridge University. His appointment in 1923 as Research Assistant marked the beginning of a long period of distinguished service in the School of African Studies at the University of Cape Town where he was appointed Senior Lecturer in Social Anthropology in 1927 and Senior Lecturer in Ethnology and Archaeology in 1934 and elevated to the status of Associate Professor in 1954 in recognition of his outstanding academic achievements and original contributions and research.

Soon after his appointment at the University he began also to take a great interest in the South African Museum and, in collaboration with the then Director Dr. E. L. Gill, undertook the sorting and arrangement of the study collection of stone implements in the Archaeology and Ethnology Department which he served as Honorary Curator from 1931 to 1956. Through his energy and enthusiasm also many additions were made and more attractive and instructive public displays were prepared for the Museum, but his last and most ambitious plans were, unfortunately, never carried out because of financial stringency.

Goodwin entered the field of South African archaeology at a time when the subject was still in its early stages of development. Although a large number of sites had been discovered during the preceding three-quarters of a century and a large amount of material had been collected, general relationships and positions in the time scale had not been determined. Goodwin immediately applied his scholarly mind and great analytical powers toward a systematic classification and chronological arrangement of the considerable collections of stone artefacts in museums and private collections. Realizing the unsuitability of earlier proposed terminologies, he applied strictly reasoned arguments based on scientifically established stratigraphic, typological, and technological evidence toward the establishment of an essentially South African cultural succession and terminology. He it was who first envisaged three major divisions of the Stone Age, an Earlier, a Middle, and a Later Stone Age, each embracing a number of Cultures and Variations. These ideas, first tentatively presented in a series of articles in the *Cape Times*, became the foundations of South African prehistoric classifications that won the support of his two eminent colleagues, the late Professor C. van Riet Lowe and the late Dr. Neville Jones.

As soon as their validity was accepted these classifications were published in a series of papers in the *South African Journal of Science* and in *The Stone Age Cultures of South Africa* in which he, in collaboration with van Riet Lowe, provided basic systematic descriptions of South African cultures. The South African successions and terminology which he then introduced remain essentially unaltered today; the terminology, moreover, has been adopted far beyond the boundaries of the Union, even as far north as the Horn of Africa.

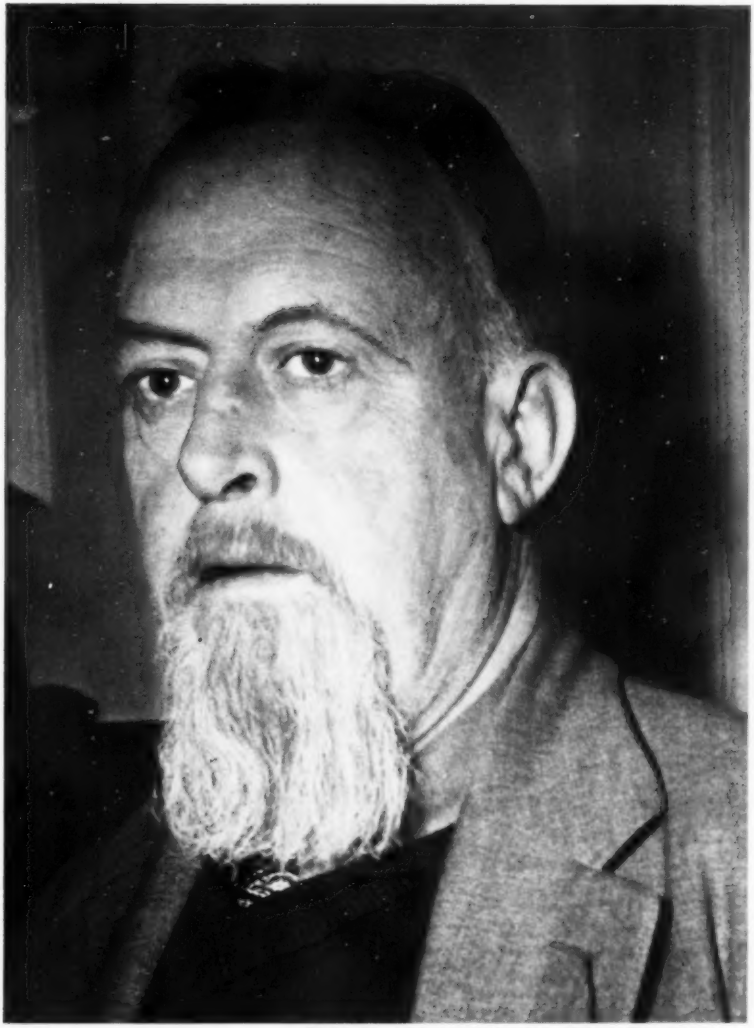
It was Goodwin more than any other who in those pioneering days stressed the need for strictly scientific methods of fieldwork and the importance of convergent lines of evidence in support of any conclusions. This disciplined approach was evident in many of his own contributions and was later embodied in his handbook on *Method in Prehistory* which has been widely acclaimed. Among his own field studies special mention may be made of the excavation of the Cape St. Blaize Cave at Mossel Bay, the important investigations of the Oakhurst shelter near the Wilderness, the study of rock-engravings at Vosburg, and his work in the caves of the Cape Peninsula. His description of the Saldanha site first drew scientific attention to the archaeological, as distinct from the palaeontological, value of the site. Latterly, his attention was focused on more recent archaeological periods when the Government of Nigeria invited him to excavate and study important deposits in that territory.

Not only did Goodwin set an example in his own research and writings but through his teachings he encouraged and trained his successors. His keen, critical scrutiny was never dogmatic nor did it overshadow his kindly personality; his readiness to listen to another's point of view and to revise, when necessary, his own conclusions encouraged free discussion. Possibly one of his greatest contributions was his influence in clarifying and canalizing the work and enthusiasm of the many amateurs by his lead in founding the South African Archaeological Society and in his editorship of the *Bulletin*. This publication provided a most successful medium of expression for the amateur and professional alike and has probably done more than anything else to stimulate clear thinking and systematics and to eliminate muddle-headedness and romantic antiquarianism.

His warm, sympathetic attitudes and diverse interests that made him a delightful companion on field-trips were also reflected in his approach to archaeology. His was never the narrow one of the taxonomist and he never lost sight of the human mind that lay behind the material remains that are so often all that the archaeologist has upon which to build. His lectures were directed toward presenting prehistoric culture in the light of the environment in which it evolved and he never let one forget that a stone tool was the work of a human being and not just a culture fossil.

He is survived by his widow and two children to whom we extend our sincerest condolences.

K.H.B.
J.D.C.
B.D.M.
A.M.T.



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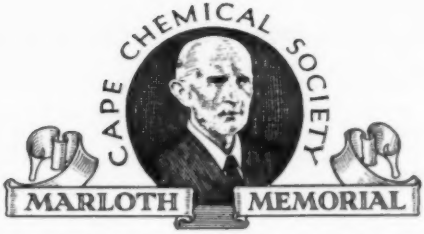
ASTLEY JOHN HILARY GOODWIN

Fellow of the Royal Society 1930–1959



HAEMATOCOCCUS IN SOUTHERN AFRICA

By M. A. Pocock

(With 6 text figures and Plates I and II)

(Read March 18, 1959)

Knowledge of the genus *Haematococcus* is briefly summarized, the existence in Southern Africa of many habitats suitable to the alga indicated, and the occurrence of the various species recorded.

The cosmopolitan species *H. lacustris* is widespread. In addition two new species are described: *H. capensis* with several varieties and forms already recorded from many localities in both the Western and Eastern Provinces and *H. zimbabwiensis* as yet known only from Zimbabwe.

The life history of *H. capensis*, described in detail, is closely comparable with those of *H. buetschlii* and *H. droebakensis*. Material of the latter collected at Stonehenge has been kept in culture at the Cape and in Grahamstown for many years. In all three species sexual reproduction is by means of gametes formed in motile cells, and asexually formed resting cells are rare.

Special attention has been given to the behaviour of *H. lacustris* from numerous sources grown under varying conditions; the dominance of the motile cell in building up the population in this as in the other species is stressed. Reproduction by means of asexually formed non-motile cells, occasional in the other species, has developed to such a degree in this species that it has largely replaced sexual reproduction which is comparatively rare, the gametes being formed not in motile cells but on germination of asexually formed resting spores. Gametes and zygotes have been found once in nature near Grahamstown.

Partial success in applying Peeble's method for inducing sexual reproduction has yielded indications of the existence of two types of naked swarmers: the smaller, gametes which conjugate to form zygotes, the larger of the nature of accessory cells. These develop without conjugation, forming resting spores which eventually germinate producing normal motile cells.

The taxonomy of the genus is discussed, the combination *Haematococcus lacustris* (Girod.) Rostafinski is maintained and the division of the genus into two genera proposed by Droop considered. Careful comparative studies of the species leads to the conclusion that such a division is not justified and the genus *Balticola* proposed by Droop is therefore rejected. A key to the species of *Haematococcus* is included.

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INTRODUCTION

The genus *Haematococcus* Agardh is best known from the cosmopolitan species *H. lacustris* (Girod.) Rostafinski* (*H. pluvialis* Flotow). Based on the characters of that species the genus was distinguished from *Chlamydomonas* by (1) the widely outstanding membrane connected with the central protoplast by numerous delicate colourless protoplasmic strands and (2) the vacuolated parietal protoplast containing many pyrenoids

The discovery and description by Blochmann (1886) of a second species, *H. buetschlii*, necessitated a modification of the generic diagnosis since this species differs from the first chiefly in (*a*) the coarse protoplasmic processes into which the chloroplast extends, these in turn being connected with the membrane by delicate thread-like protoplasmic strands, and (*b*) the presence of two pyrenoids only, one anterior, the other posterior to the central colourless region in which the nucleus lies. In its life history too this species differed considerably from *H. lacustris*. Blochmann found his species in rain water which had collected in two stone basins '*im Schlossgarten zu Schwetzingen*' near Heidelberg. He kept it in culture for some time, making careful observations the results of which are embodied in an admirably clear, well-illustrated paper dealing with nearly every phase of its life history. Although distinctive, the species has rarely been found since and is as yet not known outside the Rhine Plain.

Some years later Wollenweber (1907, 1908) discovered and described a closely allied species differing from *Haematococcus buetschlii* in certain minor details of structure, particularly the form of the anterior apical protrusions, but agreeing with that species in the nature of the protoplast, more particularly in the number and position of the pyrenoids, as well as in its life cycle. Wollenweber (1908, p. 244) found his species in shallow rain-water pools in hollows of granite rocks on certain islands off the coast of Norway and named it *Haematococcus droebakensis* after the neighbouring town of Dröbak on the Christiania Fjord. His first paper was followed in 1908 by a much longer one dealing with a number of forms and varieties of his species and including detailed studies of the life cycle, not only of it but also of *H. lacustris*; like Blochmann, Wollenweber makes a point of including numerous drawings of all the forms dealt with. His paper is remarkable for the emphasis laid on the need for the establishment and study of pure cultures for comparison with the alga as found in Nature.

PART I

Ecological Aspects in Southern Africa

A great part of the land surface of southern Africa is occupied by geologically ancient rocks, outstanding among them being the hard quartzites of the Table Mountain Sandstone (T.M.S.) and Witteberg Series, the doleritic dykes and sills

* For the most recent accounts of the synonymy of this species see Smith (1950) and Droop (1956). The generic name *Sphaerella*, recently of general use among British and American algologists but never accepted by Continental workers, has been shown to be inapplicable (Smith, p. 110); Droop's argument for rejecting Girod-Chantran's specific name *lacustris* appears to me ill-founded, and I therefore agree with Smith in accepting Rostafinski's combination. (See later discussion.)

intrusive in the somewhat less ancient Karoo System, and the granites which occur in many parts. At the Cape these are mainly intrusive in the Malmesbury Series, while in Southern Rhodesia wide areas are dominated by granitic formations of the fundamental complex. The latter may form high kopjes or flatter rounded outcrops of considerable extent.

In general, the land surface as a whole is a very ancient one, characterized by extensive wind erosion, and it is usual to find the hard rocky outcrops pitted with many small depressions in which rain water collects, forming shallow pans or even fairly extensive deeper pools. Such rain-water pools form the characteristic habitat for species of *Haematococcus*, and wherever they are found—Scandinavia, many mountainous regions of other European countries, America, southern Africa—one may expect to find these algae, together with a limited but very characteristic fauna and flora typically associated with *Haematococcus*. The duration of such rain-water pools is however often brief and is quite obviously a function of their size and of local climatic conditions; the biology of their populations is consequently closely adapted to the peculiarities of the habitat.

In South Africa, in particular in the south-west Cape, rain is often followed by warm sunny days frequently accompanied by wind, and the shallower pools dry up exceedingly quickly, to be formed again by the next shower of rain—the whole cycle recurring repeatedly in a single season. Obviously only those organisms can survive which are capable of withstanding such rapidly changing conditions. Where green algae are concerned this implies the ability to pass rapidly through all stages in their life cycle, culminating in the formation of resistant resting spores, before the pool they inhabit has dried up. In the case of *Haematococcus lacustris* this is achieved by the very efficient and rapid mode of formation of resting spores by the rounding off of the motile cells followed by the secretion of a wall; the formation of haematochrome, accompanied by oily substances, helps to render such cells immune to desiccation. In the other species the same end is reached by the production of sexually formed zygospores, the whole life cycle being completed with extraordinary rapidity. Asexual resting spores, if present in these species, are comparatively rare.

The Species of *Haematococcus*

Of the species of *Haematococcus* dealt with here, the cosmopolitan *H. lacustris*, a new species from the Cape Province to which the name of *H. capensis* has been given, and one from Rhodesia, *H. zimbabwiensis*, are indigenous; *H. droebakensis* has not yet been found in South Africa but material collected at Stonehenge in Wiltshire in 1950 has been kept in culture in the Botany Departments of the University of Cape Town and Rhodes University for some years. Unfortunately it has not yet been possible to obtain material of the remaining species, *H. buetschlii*. Since there is some doubt as to the specific distinction of this species from *H. droebakensis* it is highly desirable that comparative studies of the two should be made and for this it is absolutely essential that material of *buetschlii* from the type locality at Schwetzingen should be obtained.

Inception of the Investigation

The attention of the writer was first drawn to *Haematococcus* in May 1933 during an investigation of rain-water pools on the granite outcrop known as De Klip on the Cape Flats, some 8 miles from Claremont—so called because it was the largest of the few outcrops of rock in miles of sandy flats. Every rainy season a number of comparatively large pools formed adjacent to the several granite bosses which constituted the 'rock'; once formed, these pools might last for a few weeks before drying up only to refill during the next rain, or they might last throughout the wet season, in this case the winter months. But in addition to these larger pools, whenever it rained numerous shallow depressions on the granite bosses themselves collected water so that small saucer-shaped rain-water pools were dotted over the granite. Some of these were very evanescent, lasting only a few hours, others, somewhat deeper, were longer-lived, surviving for days or even weeks according to climatic conditions; further, after each disappearance the majority filled up again in the next shower of rain, the whole process—formation, duration, drying-up and refilling—recurring repeatedly throughout the winter months.

Whereas the larger pools, on sandy loam adjacent to the granite bosses, produced a wealth of both algal and animal life, these little pans on the granite appeared at first sight barren; but when water from them was collected and concentrated in a small plankton net (often only possible by dipping up the water with a spoon), it was found that some of them produced a very characteristic if limited fauna and flora. On May 13, 1933, two days after rain, in one of these pans, some 2 by 3 feet in size and at most 3 to 4 inches in the deepest parts, a motile unicellular alga, already in all stages of development, was collected. This proved to be a species of *Haematococcus* but one which differed from all the three previously described species in the presence in each cell of one pyrenoid only. This at once distinguishes it from *H. lacustris* with its numerous pyrenoids and from the two species *H. buetschlii* and *H. droebakensis*, each with two pyrenoids, which it otherwise closely resembled both in structure and life history. The single pyrenoid proved to be a very constant feature, amply justifying the description of the alga as a new species. In the twenty-five years during which this species, *Haematococcus capensis*, has been studied, remarkably few individuals with more than one pyrenoid have been seen, except where particularly well-nourished cells preparing to divide are concerned. In such cells one or two smaller pyrenoids are occasionally seen near to the primary pyrenoid. A similar phenomenon is of even more frequent occurrence in *H. droebakensis* where secondary pyrenoids may appear near the two primary pyrenoids, also in older cells about to divide (fig. 5, C and E).

As in the case of the Norwegian species, the nature of the habitat—a very small pool of only a few days' duration, but reforming after every shower of rain—made it peculiarly suited to investigation, since the life history, completed in so short a period, was recapitulated several times in every rainy season—at least as often as the pool dried up and refilled. Even when first examined on the morning it was collected, many cells were already dividing to form daughter cells, and by the evening further examination of the morning's sample showed many cells with mature daughters as well as numbers which were reproducing sexually. This small pan was kept under

observation throughout the rainy season of this and the two succeeding years. The other pans on the granite bosses were examined from time to time, but of them all in one only, on the other larger granite boss, was *Haematococcus* ever found, whereas every time the original pan reformed it could be relied on to produce a fresh crop of the alga.

Rock-pools in other localities were examined from time to time with varying success. In 1934 and again in 1941 both *Haematococcus lacustris* and *H. capensis* were collected in pools on a rocky outcrop between Mamre and Darling; in 1935 *H. capensis* was found in one or two 'dassie reservoirs' on Kalk Bay mountain while a number of other such small pools were unproductive. Observations were interrupted in 1936 and the first half of 1937 by absence in England, during which period a preliminary account of the new species was read before the Linnean Society of London (Pocock, 1937). Towards the end of 1937 quarrying had begun at De Klip and although the two larger bosses were still untouched, it was painfully obvious that they too would soon go. It therefore became imperative that other localities for *H. capensis* should be found. The alga might be expected wherever rock-pools such as those described above occur, and the probability was that it would prove to be widespread in South Africa. But the very nature of the pools and the close adaptation of the alga to its habitat makes its collection very much a matter of chance. A rich growth of motile cells may continue present in a pool for several days, but more usually an outburst of activity culminating in extensive sexual reproduction is followed within two or three days by the complete disappearance of *Haematococcus* from the plankton. In such cases the alga does not reappear until after the pool has dried up and been refilled. Hence for a given pool the right time after rain, in the conditions existing at the time, must be found; if, as in the case of the original De Klip pan, the pool in question has been kept under observation for some time, it is of course possible to prognosticate the state of the algal vegetation with a fair degree of accuracy. Collection in such small pools is not easy and undoubtedly they have been neglected. Furthermore, on one day *Haematococcus* may be present in a given pool but not in a number of adjacent pools which are apparently no different. For example, on September 6, 1937, a special excursion was made to the south end of the Cape Peninsula to try to find an undisturbed and easily identifiable locality for *Haematococcus capensis*. On the western slopes of Red Hill behind Simonstown eleven apparently suitable rock-pools were examined, in none of which were any motile cells found, although one yielded a few resting cells, probably of *H. lacustris*. A few miles to the south-west, however, on the lower slopes of the Bonteberg, four very shallow pools on quartzite were examined, and while two of these also were barren, the other two contained good growths of *H. capensis*. The motile cells were exceptionally large but with attenuated and much vacuolated protoplasts (fig. 1, C and D), possibly due rather to low temperature than to actual paucity of nutrient salts. In the laboratory with addition of culture solution the protoplasts soon began to assume the normal size and shape although they remained more vacuolated than usual (fig. 1, E). Subsequently other localities have been found, but none comparable in ease of access and certainty of identification and of the presence of the alga to the original De Klip pan. To date, the locality most closely

approximating to the desired qualifications is a koppie above Buffels Bay. Since this is within the Cape Point Nature Reserve there is a reasonable hope that it will not be destroyed.

The alga developed rapidly in the pan in which it was originally found at De Klip. Within twenty-four hours of the formation of the pool it was already abundant and passed through all the stages of its development within two or three days. The almost complete absence of other motile green algae in the pan made it ideal for studying structure, variations in form and life history. Unfortunately in 1939–40 the quarrying operations reached this particular boss and where it formerly stood there is now a deep hollow permanently filled with water to a depth of many feet.*

Quite recently (December 1957) pools on an extensive flattish granite outcrop at Zimbabwe in Southern Rhodesia were examined and a small quantity of a *Haematococcus* with two pyrenoids was collected. It was at first thought to be *H. droebakensis* but closer examination revealed certain small but significant differences which are judged sufficient to warrant its description as a new species, now named *Haematococcus zimbabwiensis*. The pools were obviously very newly formed—the first rains had fallen on the two previous days—and the material was unfortunately scanty. Search in several other localities was unrewarded, and at present the material though sufficiently distinctive to warrant description is not sufficient to determine whether it too, like *Haematococcus droebakensis* and *H. capensis*, will be found to include a series of comparable varieties and forms. In all probability further search, possibly later in the rainy season, will result in this species being found in many similar localities in Rhodesia and adjacent territories.

Description of the Species

I. *HAEMATOCOCCUS CAPENSIS* sp. nov.

Figures 1 and 2. Plate I, B–G; Plate II, A–M

Cellula subglobosa, ellipsoides aut piriformis, polus anticus rotundatus vel aliquando cum papillis clitelliformibus duobus, polus posticus late rotundatus aut in apiculum conicum productus. Protoplasta elongata piriformis crassis radiantibus

* De Klip and its immediate surroundings formed a unique botanical treasure house. Besides the *Haematococcus capensis* pan, the larger pools formed annually adjacent to the granite bosses produced a wealth of rare and interesting algae—*Volvulina*, *Astrephomene*, *Volvox*, *Hydrodictyon*, many motile unicells, some still undescribed, Desmids, *Oedogonium*, *Spirogyra* spp.—as well as abundant animal life—Streptocephalids, *Flosculariа*, and many other species. In the crevice of the granite grew succulents, bulbous plants such as Oxalids, *Spiloxene*, *Asparagus* spp., while in the damp patches of grass between the boulders *Isoetes stellenbosiensis*, an undescribed species of *Oedocladium*, aquatic *Crassulas* and so on could be found. In 1937 a recommendation from the Linnean Society of London that the site should be preserved as a small nature reserve (Pocock, 1937, p. 580) was ignored. The granite has been quarried by the Cape Divisional Council for road metal and at the time of writing (January 1959) most of the bosses have been quarried away to a depth of a dozen feet or more while the rest of the area has been used for machinery, shacks, pig-styes, etc. Thus what was once a landmark on the Cape Flats of considerable historic interest in addition to its botanical and zoological value has been destroyed—another sacrifice to the immediate needs of a utilitarian age.

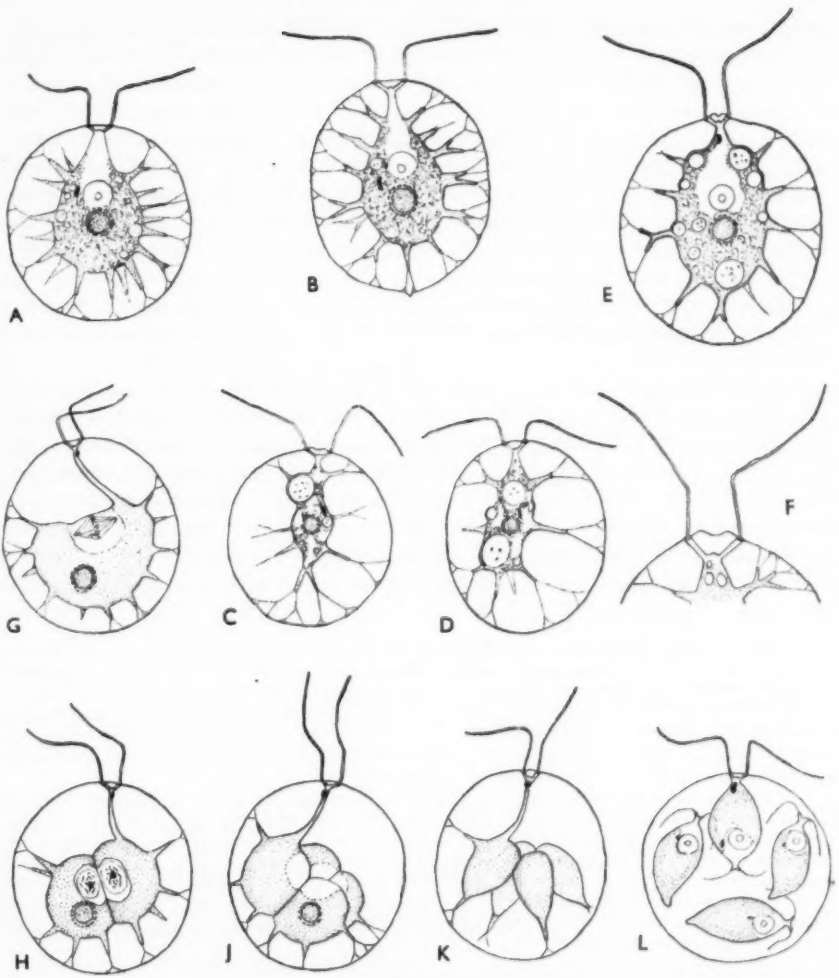


FIGURE 1. *Haematococcus capensis* var. *capensis*. Figures C–F from the Bonteberg, the rest from De Klip.

A–F, structure of the cell: A, B, typical mature cells, B with posterior pole apiculate; C, D, cells with attenuated, much vacuolated protoplast from large shallow pools at the Bonteberg in cold weather; E, another after a day or two in culture solution in the laboratory; cell of maximum size, protoplast still vacuolated but size normal in proportion to that of the cell; F, anterior pole from a similar cell showing bilobed papilla, flagellary tubes with emerging flagella, branched processes and three apical vacuoles. G–L, Asexual reproduction: G, metaphase of first division, showing change in shape of protoplast, axis of plane of division very slightly inclined to polar axis of the cell; H, preparation for second division, plane of first division inclined at a slightly greater angle; J, K, completion of second division and organization of the zooids; L, zooids separated, flagella lengthening, stigmas present but pyrenoids not yet apparent; anterior zooid with 2 stigmata, two sets of flagellary apparatus. F ×1,000, the rest all ×500.

projecturis, in quae chloroplasta extenditur, hae filis tenuibus incoloribus protoplasmicis cum membrana externa sunt conexae: polus anticus in processum incolore rostriformem producta, hic paene ad murum extendens, tunc structuras duas breves tubulatas paene ad rectum inter se angulum edens, per quas duo flagella ex adversis papillae apicalis partibus emergunt: flagella plus minusve duas partes cellulae longa: chloroplasta fere pelviformis in processus protoplasmicos extendens, saepe cum multis inclusionibus et vacuolis granula moventia continentibus: color subviridis, in densa basica parte pyrenoides una grandis immersa: stigma elongatum conspicuum, aliquando aequatoriale aliquando in rostro antico: nucleus centralis aut centro paullo anterior: haematochroma nullum.

Reproductio: cellula motilis manet donec corpora reproductiva plene formantur. Asexualis reproductio a duobus usque ad octo (rarius sedicim), quattuor plerumque zooides biflagellatas.

Sexualis reproductio a 32 (rarius 8 vel 16) usque ad 256 (rarius 512) cellulis in cava sphaeroide formatis quae cum maturae sunt separantur ad gametes biflagellatas minutissimas fusiformes formandas, quae gametes cum pullulaverunt parentem vesiculam rumpunt et in aquam circumjacentem ubi effugerunt, ibi binae conjugantur planozygotes quadriflagellatas formanturae: zygotes post activum temporis spatium residet rotundatur zygosporum cum muria laevibus format. Germinatione ex zygosporo fiunt zoospori quattuor aliquando plures.

Videntur aliquando sporae quiescentia grandis rotundae haud multo haematochromate.

Cell subglobose, ellipsoidal or pyriform: anterior pole rounded or sometimes with two papillae forming a saddle-shaped apex: posterior pole broadly rounded or drawn out into a conical apiculus. Protoplast elongated pyriform with thick radiating protrusions into which the chloroplast extends and which are connected with the outer membrane by fine colourless protoplasmic strands: anterior pole drawn out into an elongated colourless beak which reaches nearly to the outer wall, then gives out two short tubular structures almost at right angles to one another through which the two flagella emerge on opposite sides of the apical papillae when present.

Reproduction: Cell continues motile until the reproductive bodies are fully mature. Asexual reproduction by from two to eight, rarely sixteen, most often four, biflagellate zooids. Sexual reproduction by from 32 (occasionally 8 or 16) to 256 (sometimes 512) cells, formed in a hollow spheroid, which when mature separate forming minute spindle-shaped biflagellate gametes; these move actively for a short time within the parent vesicle then rupture it, escape and swarm actively in the water, soon conjugating in pairs to form quadriflagellate planozygotes; after a period of motility the planozygote settles down and rounds off to form a smooth-walled zygospore. On germination the zygospore gives rise to four or more zoospores.

Large round resting spores with some haematochrome occasionally seen.

Dimensions

Cell	$(14-)31-58(-71)\mu \times (26-)40-66(-84)\mu$
Protoplast (without processes)	$(9-)13-24\mu \times (13-)22-58\mu$
Flagella	$22-35\mu$
Daughter cells, number	2, 4, 8, rarely 16, most often 4
Daughter cells, mature	$9-13\mu \times 18-22\mu$
Gametes, number	very occasionally 8 or 16, normally 32–512
Gametes, size	$3-5\mu \times 4-9\mu$
Planozygote	$4-6\mu \times 7-10\mu$
Zygospore (newly formed)	$4-8\mu$
Zoospore, just liberated	$14-28\mu \times 28-56\mu$, most often $14-17\mu \times 28-31\mu$

Geographical Distribution. So far, found from the Cape Peninsula northwards to Namaqualand and eastwards to the Amatola Mountains. Probably widespread, to be expected wherever hard wind-eroded rocks outcrop and rain water collects on them to form small pools.

Occurrence. Western Province: Small rain-water pools on granite or quartzites in the Cape Peninsula, especially in the south Peninsula. Original (type) locality a small pan on granite at De Klip (now destroyed); pools on granite at De Klip Junior, on road from Busy Corner to the Strandfontein Road. 'Dassie reservoirs' in quartzite, Table Mountain, Muizenberg and Kalk Bay mountains; pools on quartzite on kopje above Buffels Bay (type locality),* shallow pools on quartzite on lower Bonteberg, and outside boundary of Cape Point Nature Reserve. On granite: Paarl Rock; Mamre; rock-pools near Darling; pools on granite at Grootberg between Hondeklip Bay and Kamieskroon, Namaqualand. All during the rainy season, May to October. *Eastern Province:* Grahamstown, pools on Witteberg quartzite, Mountain Drive; on shales or mudstones of the Karoo Series, outcrop above pan, Cradock Road, 6½ miles from Grahamstown; pools in outcrops in river bed, Palmiet River, Table Hill Farm, 7 miles from Grahamstown. Pools in dolerite sill, Rockford, near the Hog's Back, Amatola Mountains.

HAEMATOCOCCUS CAPENSIS var. *CAPENSIS*

Figures 1, 2, A–M; Plate I, B, C, E, F; Plate II, D–G

Cellula subglobosa, polus anticus aut rotundatus, aut papilla parve bilobata, polus posticus quoque rotundatus aliquando apiculo minimo.

Cell subglubose, anterior pole rounded or with a small bilobed papilla, posterior pole broadly rounded, occasionally with a very small apiculus.

Type: Figure 1, A, B.

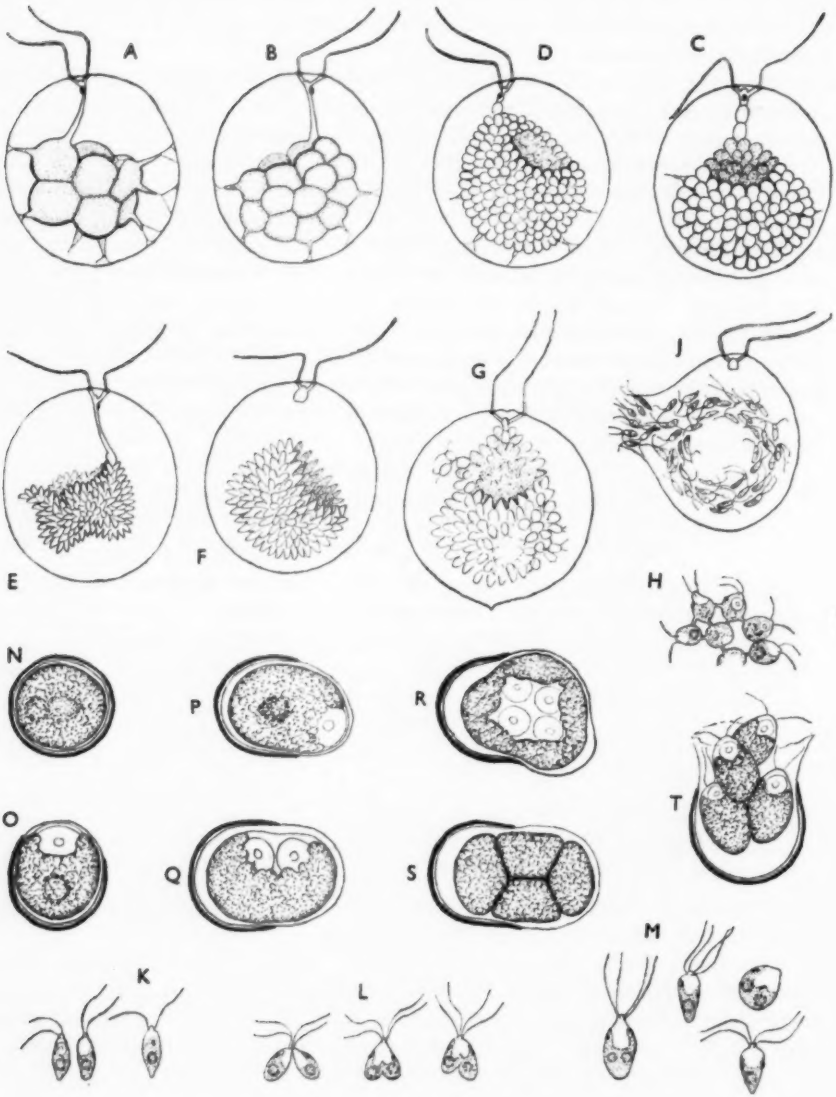
Dimensions

Cell	$24-62(-71)\mu \times 27-66(-75)\mu$
Protoplast (without processes)	$15-24\mu \times 24-53\mu$
Mature sexual cell	$18-60\mu \times 22-62\mu$
Mature sphaeroid of gametes	$13-40\mu \times 26-44\mu$
Gametes, most often	$3-4\mu \times 5-6\mu$
Planozygote	$4\mu \times 8-9\mu$
Zygospore, germinating	$31-35\mu \times 33-41\mu$

Geographical Distribution. Chiefly within the Cape Peninsula, occasional to the north thereof; rare in the Eastern Province (as yet only one record).

Occurrence. Western Province: De Klip; De Klip Junior (occasional); 'dassie reservoirs' in quartzite, Table Mountain, Kalk Bay (Harrison) and Muizenberg mountains; Bonteberg; small rock-pool near Cape Point Reserve boundary; koppie above Buffels Bay (type locality); Paarl Rock; Mamre, granite boss in centre of village; rock-pools on Malmesbury shales between Darling and Yserfontein. *Eastern Province:* Pools in dolerite sill, Rockford, Hog's Back.

* The original locality having been destroyed, the Buffels Bay kopje is designated as a typical locality; it is readily identified, comparatively easy of access, and, since it is within the Cape Point Nature Reserve, unlikely to be destroyed, at any rate in the near future.


A
B
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HAEMATOCOCCUS CAPENSIS var. 2, var. *TORPEDO* var. nov.

Figures 2, N–T; 3, A–S. Plate I, D; Plate II, A, B, H–M

Cellula elongatellipsoides, paene oblonga, si seces; lateribus aequatis, polus anticus papillatus aliquando rotundatus; polus posticus aut rotundatus aut apiculatus aliquando. Protoplastae latera aequata, proxima membranam externam, paucis aut nullis processibus protoplasmicis; processus protoplasmici prope polum anticum breves, ad polum posticum longiores et adulti.

Cell elongate-ellipsoid, almost oblong in lateral optical section, posterior pole rounded or slightly apiculate; sides of protoplast flattened very close to outer membrane, with few or no protoplasmic processes; protoplasmic processes near anterior pole usually short, at posterior pole longer and well developed.

Type: Plate II, A, B.

Dimensions

Cell	$13-31\mu \times 44-84\mu$
Protoplast (without processes) .	$12-24\mu \times 31-58\mu$
Flagella	$22-35\mu$

Geographical distribution. As yet found only on the South Peninsula.

Occurrence. Originally found in a 'dassie reservoir', a pool in a small deep hole (about 12 in. diameter and 9 in. deep) in quartzite on the top of a kopje above Buffels Bay (Type locality), June 17, 1939, and again in November, 1941, not found in any other pools but kept in culture. In July 1958 the vegetation had altered and the original pool could not be located, but the variety was collected in several pools, while in others var. *capensis* was present. A week later, both were present in most of the pools as well as *H. lacustris*. Not as yet found anywhere else.

HAEMATOCOCCUS CAPENSIS var. 3 var. *PIRIFORMIS* var. nov.

Figure 4, A–H; Plate II, C

Cellulae paries piriformis, polus posticus angustior quam anticus, obtuse fastigatus; protoplastae processus posticus adulti qui in umbonem membranae posticae plerumque extenduntur. Plerumque minor quam var. *capensis*.

←

FIGURE 2. *Haematococcus capensis* var. *capensis*. Sexual reproduction.

A, 8-celled and B 32-celled stages. C, D, division nearly complete showing different positions of the pore. E, F, constituent cells changing from globose to fusiform, globoids about to break up; in F the globoid has broken away from the small apical cell which is still attached to the parental flagella. G, separation of the gametes beginning, flagella of some partially detached gametes visible. H, portion of G more highly magnified to show protoplasmic connexion between the cells and developing flagella, shape of cells not yet finally altered. J, globoid disintegrating, membrane ruptured and some of the gametes already liberated, the remainder still very active within the membrane, some escaping. K, swarming gametes, L, conjugation in process, and M, zygotes, 3 still motile (planozygotes), one at rest beginning to round off.

Haematococcus capensis var. *torpedo*. N–T Germination of the zygospore.

N, enlargement complete, nucleus central with large pyrenoid adjacent to it. O, vesicle beginning to form, nucleus in colourless anterior apex. P, vesicle enlarging, protoplast passing into it, leaving a space at the posterior pole. Q, nucleus lateral, first division complete, R, polar view of vesicle showing four nuclei still apical in position. S, cytoplasmic cleavage complete but parts still in close contact. T, vesicle ruptured, zoospores about to escape, no wall, flagella short, nucleus apical.

H, K–M $\times 1{,}000$; the rest all $\times 500$.

Cell pear-shaped, posterior pole narrower than anterior, bluntly fastigate. Posterior processes of protoplast well developed extending into the posterior protuberance of the membrane. Usually smaller than var. *capensis*.

Type: Figure 4, A–D.

Dimensions

Cell	$15-31\mu \times 26-44\mu$
Protoplast (without processes) .	$9-13\mu \times 13-26\mu$
Sexual cell	$19-29\mu \times 22-40\mu$

Geographical distribution. Most widely distributed of all the varieties. In the Western Province occasional, mixed with var. *capensis*, but in the Eastern Province is by far the commonest and usually the only form found.

Occurrence. Western Province: De Klip, occasional, mixed with var. *capensis*; more abundant at Mamre and between Darling and Yserfontein. *Eastern Province:* Grahamstown, narrow but rather deep pool in Witteberg quartzite on mountain path above Somerset Street (Pl. II, C); tiny pool on isolated quartzite boulder farther up the same path; Signal Hill; Cradock Road, 6½ miles from Grahamstown, pools in rocks above pan; hollows in rock in bed of Palmiet River, Table Hill Farm, and in large rocks lower down the stream (Type locality; Rockford, near the Hog's Back, Amatola Mountains, pools in dolerite sill).

HAEMATOCOCCUS CAPENSIS var. *PIRIFORMIS* forma *CAUDATA* form. nov.

Figure 4, J, K

Polus membranae posticus in caudam manifesto tubulatam productus.

Posterior pole of membrane drawn out into a pronounced tubular tail.

Type: Figure 4, J, K.

Dimensions

Cell .	$22\mu \times 48\mu$
Tail .	$6-8\mu$

Geographical distribution. Coastal strip from Mamre north to Namaqualand.

Occurrence. Mamre; between Darling and Yserfontein; pools on granite, Grootberg, between Hondeklip Bay and Kamieskroon, Namaqualand (Type locality), with longest 'tail'.

→

FIGURE 3. *Haematococcus capensis* var. *torpedo*.

A–C, mature vegetative cells of varying forms.

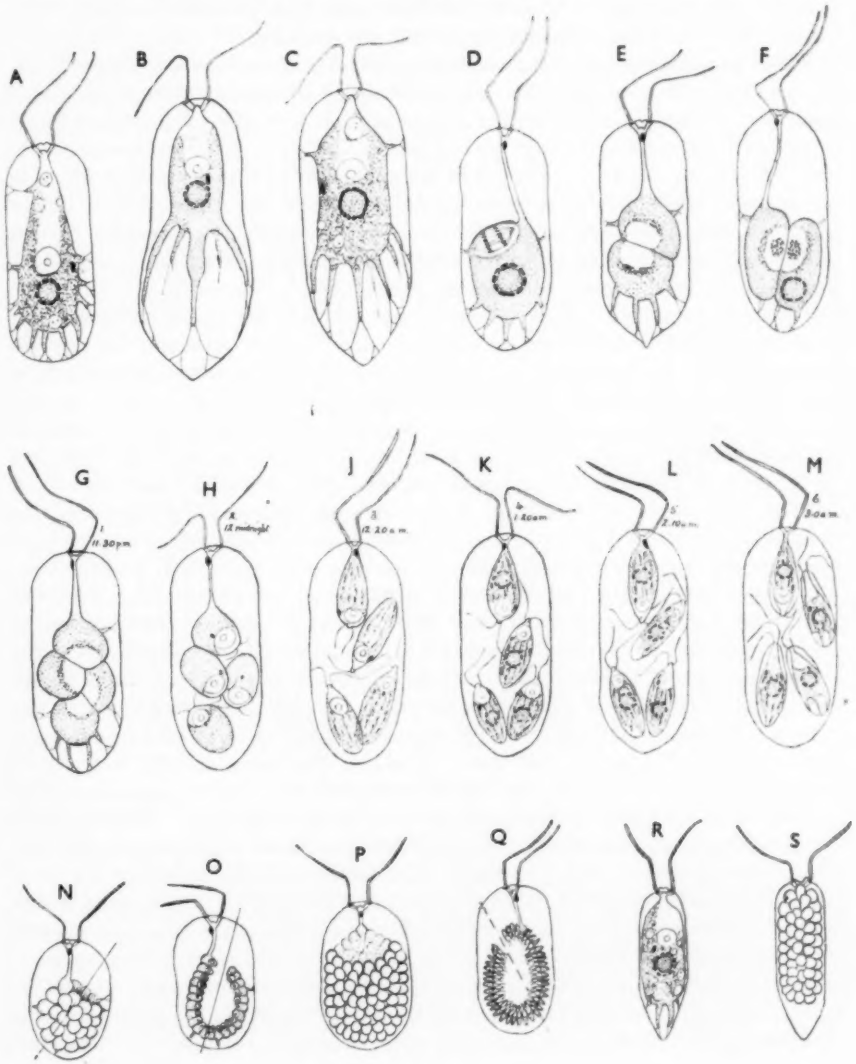
D–M, asexual reproduction: D, anaphase of first division; E, F, two-celled stage, in E zone of granules adjacent to nuclei shown, in F, metaphase of second division. Note varying inclination of plane of first division—very slightly inclined to the polar axis of the cell in D, more so in F, much more so in E. G–M, maturation of zooids in a single cell: G, 11.30 p.m., second division complete; H, 12 midnight, eyespots formed, flagella lengthening, separation of zooids beginning; J, 12.20 a.m., zooids elongated, flagella nearly fully developed; K, 1.20 a.m., pyrenoids differentiated, nucleus still apical; L, 2.10 a.m., separation of membrane beginning at posterior pole; M, 3.0 a.m., separation obvious at both posterior and anterior poles, processes visible at posterior end, nucleus beginning to pass to a central position in the cell.

N–S, sexual reproduction: N, early stage in cell division, plane of first division shown by position of pore, inclined at about 45° to polar axis of the cell; O, optical section of later stage showing inward position of the nuclei, inclination of plane of first division less, pore nearly apical; P, gamete globoid nearly filling the cell, division almost complete; Q, optical section of mature globoid, gametes elongated, fusiform, with flagella arising from inwardly directed apices; R, S, small sexual cells, S with nearly mature gamete globoid.

All ×500.

Notes on the Distribution of the Various Forms of *Haematococcus capensis*

Of the various forms of *Haematococcus capensis* the typical form, var. *capensis*, is characteristic of the mountainous regions of the Cape Peninsula, particularly the South Peninsula; so far, apart from the single exception of Rockford in the Amatola



Mountains, near the Hog's Back, it has not been found away from the south-west Cape. It is typically sub-spherical, the anterior pole often smoothly rounded, sometimes with a very small and insignificant bilobed apical papilla; similarly the posterior pole is usually rounded, but occasionally has a minute apiculus or mucro.

The size is somewhat variable but is usually large; the curiously vacuolated material collected on the Bonteberg was nevertheless as large as any ever found, and in the warmer laboratory with added culture solution, the attenuated protoplasts soon reached the normal size although vacuolation still persisted.

The second form, var. *torpedo*, although most distinctive, has as yet been found only on the kopje above Buffels Bay; nevertheless, it is so striking and apparently such a stable form that it is judged worthy of varietal rank. The original material did well in culture and bred true through several generations. Material from liquid cultures was put on agar plates (0·75 per cent agar in Juller's culture solution) where it reproduced sexually, the planozygotes collecting round the edges of the drops of culture solution as conjugation was achieved. The zygospores were allowed to mature on the agar, then removed to culture solution where they germinated. On the agar they increased greatly in size and became golden brown in colour (fig. 2, N–T; Pl. II, D–G). From the zoospores thus obtained liquid cultures were again established; this was repeated several times and the alga kept true to form, retaining the essential characters of the cell which have given the varietal name, the narrow elongated form with a tail of protoplasmic strands extending into the posterior pole of the cell membrane strongly suggesting the streamlined form of a torpedo. Minor variations appeared in the cultures—particularly as to the presence or absence of apical papillae on the anterior pole, and the form of the posterior pole, sometimes round, sometimes more or less fastigate—but as in *H. droebakensis* such variations may apparently be found in any strain of this species, and at present it seems advisable not to attempt to segregate such minor variations. Extensive breeding experiments may perhaps show that they are indeed inherited characters which may serve to distinguish a number of minor varieties or forms. It even seems likely that more extensive investigations of 'dassie reservoirs', particularly in the south Peninsula, would add considerably to the records, not only of this very distinctive variety but to our knowledge of the species as a whole. The results of the 1958 observations have already been fruitful—it was extremely fortunate that just before the first visit on July 19 many pools had formed, either by rain or by precipitation from mist or low-lying cloud, not only in the small holes in the boulders (the so-called 'dassie reservoirs') but in more extensive shallow depressions in the flatter areas of rock on the summit of the kopje. Thus although changes in the vegetation made it impossible to recognize the original dassie reservoir, there were many more pools, practically all of which yielded one or more species or varieties of *Haematococcus*. The change in the populations of the various pools within the week between the first and second visits is also significant. On July 19 most pools revealed but a single species or variety—in most var. *torpedo*, in others var. *capensis*—while *H. lacustris* was remarkably rare. On July 29 *H. capensis* var. *torpedo* was still the commonest form, but now in most pools there was a mixture, the two varieties of *H. capensis* were both present in each pool, while *H. lacustris* was much more abundant

than on the previous visit, and in a few pools, instead of *Haematococcus*, one or two species of *Chlamydomonas* were present, while the rosy rotifer, *Philodina roseola*, almost invariably present in *Haematococcus* pools, but scarce on the first visit, was now very much more numerous. This visit has shown that var. *torpedo* is not confined to the original small pool, but occurs in most of the pools formed on this particular koppie, provided the right time is found. As a corollary to this, it may well be that it occurs in many other similar localities in the south Peninsula, and possibly other mountainous regions in the Table Mountain Series.

Haematococcus capensis var. *piriformis* appears to be much the most widely distributed form of the species. At the Cape itself it is comparatively rare and has

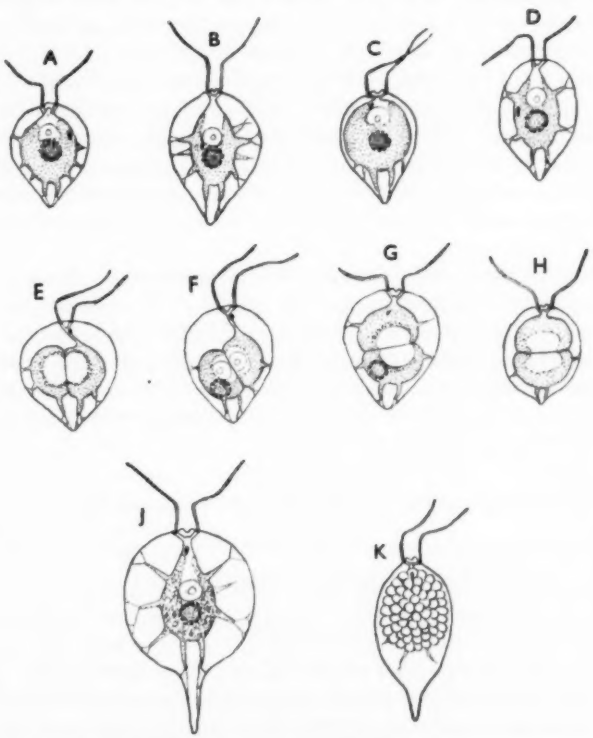


FIGURE 4. *Haematococcus capensis* var. *piriformis*.

A–D, mature cells showing variation in shape. E–H, two-celled stages, showing inclination of plane of first division varying from nearly parallel to the polar axis of the cell in E to nearly perpendicular to it in H (i.e. division apparently almost transverse), F and G intermediate; pyrenoids present in the two latter, not in E and H. *H. capensis* var. *piriformis* forma *caudata* from Grootberg, Namaqualand.

J, large mature vegetative cell, K, smaller sexual cell with nearly mature globoid of gametes.

All figures ×500.

not been found in the mountainous regions, but at De Klip it was sometimes present though usually overshadowed by the typical var. *capensis*. As one goes north it becomes commoner—at Mamre this variety and var. *capensis* occurred in approximately equal numbers; at Darling it predominated slightly while in Namaqualand at Grootberg near Kamieskroon the striking form *caudata* was the only one seen, but it must be remembered that only one collection was made. On a large granite boss near the camp there were several comparatively deep, young pools; that year Namaqualand had had a phenomenal rainfall of 8 inches (4 inches per year is considered good), and the clear pools on this outcrop were large enough to provide very welcome baths for the travellers who had had a gruelling cross-country drive from Hondeklip Bay—the unprecedented rainfall had played havoc with the gravel roads and the car had been bogged down three or four times in as many miles. Several of the pools yielded beautiful growths of the caudate form, most striking, with the main body of the cell nearly globose, then narrowing suddenly in the very characteristic tail. At both Mamre and Darling a similar form but with shorter tail was obtained. Therefore since in culture the Grootberg material, although it flourished, showed a tendency to a shortening of the tail approximating to the Mamre-Darling material, it seems best to regard it as a form and not a distinct variety. The original material did not include sexual cells so was probably a very young growth; sexual cells appeared in the cultures (fig. 4, K).

In the Eastern Province *H. capensis* var. *piriformis* is usually the only form of the species found and form *caudata* has not been recorded; *H. lacustris* is usually present with it. It is only in the mountainous region of the Hog's Back that the typical var. *capensis* has been found; further search in mountainous parts of the Eastern Province may show that at higher altitudes the latter variety once more becomes predominant.

Details of development and life history in *Haematococcus capensis* are described more fully in the next section.

II. *HAEMATOCOCCUS DROEBAKENSIS* Wollenweber 1907 & 1908

Balticola droebakensis (Wollenweber) Droop 1956 A, p. 64

Figure 5, A–G; Plate I, F, H

This species has not been recorded from South Africa but material collected in 1950 in a small pool formed in a mortice hole of one of the fallen trilithons at Stonehenge (Pocock 1951) was brought to the Cape and kept in culture for years, first in the Botany Department of the University of Cape Town, then in that of Rhodes University, Grahamstown. At the time of collecting there were very few records of the species in the literature subsequent to Wollenweber's detailed study in 1908, apart from one doubtful record from Wales (West and Fritsch, 1927, p. 79). Since that time, however, it has been recorded from various localities in Sweden by Pringsheim (1951) and Droop (1956, p. 54) who also collected it in Finland (Droop, p. 23). It is probably widely distributed in mountainous regions of northern Europe.

The Stonehenge material proved very tolerant of culture conditions but, whereas in the original material most of the cells were broad and subglobose with a few of a

narrower form close to Wollenweber's var. *fastigatus*, in the later cultures in South Africa the latter form usually predominated. The number of pyrenoids, too, varied considerably. In young growths there were regularly two, one anterior, the other posterior to the central vacuole; with age however there was a strong tendency to an increase in the number, but always in two groups, and the secondary pyrenoids were usually distinguishable from the original ones by their smaller size (fig. 5, C). The chloroplast is very distinctive, consisting of anterior and posterior massive portions containing the pyrenoids, these two polar portions being connected by a very thin parietal girdle so that the central region of the cell appears very pale green, almost colourless. The nucleus usually lay in the lower part of the colourless central region, somewhat eccentrically (fig. 5, A–D). As in the other species the position of the eyespot was very variable, depending mainly on the stage of development of the cell—during cell division it moves up towards the anterior apex of the protoplast. As indicated above both the typical form and var. *fastigatus* were present in the material, the latter apparently reacting more favourably to culture conditions than the former. The anterior pole usually bore a minute bilobed papilla, but this was not always discernible.

In 1950 a number of pools on granite islets in Oregrund Sound were examined, but although *Stephanosphaera* was collected—extremely young colonies only—no *Haematococcus droebakensis* was found. Since then, however, it has been obtained from this region (Droop, 1956).

Dimensions of the Stonehenge Material

Cell	38—40μ × 40—47μ
Protoplast (without processes) .	22—30μ × 30—41μ

Geographical distribution. Northern Europe: Great Britain, Scandinavia, Finland.

Occurrence. Norway: rain-water pools on granite on islands in the Christiana Sound, near the town of Dröbak (Wollenweber, 1907). *Sweden:* pools on glaciated granite rocks on the shore of the Erken Lake (Pringsheim, 1951); Oregrund (Droop, 1956, p. 54). *Finland:* Tvärminne (Droop, l.c.). *Great Britain:* Stonehenge, Wiltshire, small rain-water pool in mortice hole in fallen stone of trilithon (Pocock, 1951); Wales (West and Frutsch).

III. *HAEMATOCOCCUS ZIMBABWIENSIS* sp. nov.

Figure 5, J

Cellula late ellipsoides, membrana externa a protoplasta distans, polus membranae anticus late aequatus duobus papillis aequissimis. Protoplasta ellipsoides, polus posticus rotundatus, polus anticus brevis crassus a membrana distans, duobus tubulis flagellatis ita diversissime insertis ut utrimque ex papillis apicalibus emergant. Chloroplasta toti protoplastae praeter partem ad apiculum anticum intus obtenta, constans ex partibus densis anticis et posticis quarum utraque pyrenoidem unam magnam continet; poli duo zona tenuissima mediana parietali, in qua includitur centralis incolor vacuola, ubi est nucleus, conexae; chloroplasta extenditur in partes basicas crassas radiantium processuum protoplasmicorum a tubulis flagellatis fere circum protoplastam pariter dispositorum; partes distales processuum protoplasmi-

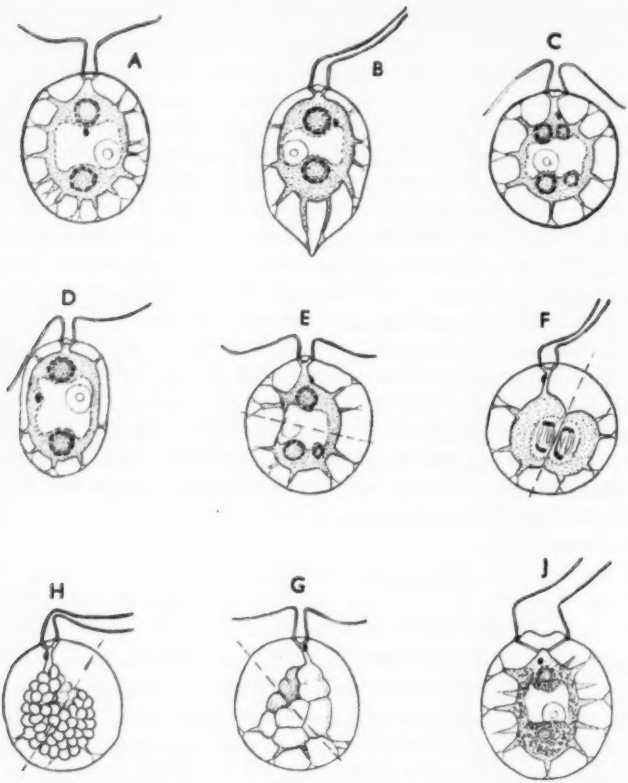


FIGURE 5. *Haematococcus droebakensis* from Stonehenge, Wiltshire. Figures A–G.

A–D, mature cells of various types: B resembling var. *fastigata* Wollenweber; C, with a secondary pyrenoid close to each primary pyrenoid. E, reorganization of the protoplast preparatory to division, anterior pole of the protoplast now lateral with nucleus just below the surface. Plane of first division will be almost transverse to the polar axis of the cell. F, late anaphase or early telophase of second division.

G, H, sexual reproduction: G, eight-celled stage, cleavage of next division just beginning; H, cell division nearly complete. Axis of plane of first division shown in figures E–H.

Haematococcus zimbabwiensis sp. nov. Figure J.

Young but nearly mature cell; many granules in cytoplasm adjacent to the central vacuole.

All figures × 500.

corum quae cum membrana externa conexae sunt filiformes, incolores, aliquando ramatae. Stigma conspicuum, prope apiculum anticem incolorem.

Cell broadly ellipsoidal, outer membrane widely distant from the protoplast, anterior pole broad with two very flat papillae. Protoplast ellipsoidal, posterior pole rounded, anterior pole short, stumpy, distant from the membrane, bearing two flagellary tubes inserted at a wide angle and emerging at

opposite sides of the apical papillae. Chloroplast lining the whole protoplast except a very small anterior part, consisting of massive anterior and posterior portions each containing a single large pyrenoid, the two polar portions connected by a very thin parietal zone enclosing the large colourless central vacuole in which the nucleus lies; chloroplast extending into the thick basal portions of the radiating protoplasmic processes spaced evenly round the protoplast from near the flagellary tubes; distal portions of these protoplasmic processes which connect them with the outer membrane thread-like, colourless, sometimes branched. Stigma conspicuous, near the colourless anterior apex.

Type: Figure 5, J.

Dimensions

Young cells:	Outer membrane . . .	39μ × 43μ
	Protoplast (without processes) .	17μ × 34μ

Geographical distribution. Southern Rhodesia.

Occurrence. Great Zimbabwe, rain-water pools on low granite outcrop between the Acropolis and Valley of Ruins, just outside the boundary fence of the Reserve. Associated with the usual 'Rosy Rotifer' (*Philodina roseola*) and a second, much larger rotifer, also rosy, but covered with long, slender, hollow, conical appendages; December 1957.

Since the first rains after a prolonged drought had fallen two days previously, followed by showers the following day, the pools could at the most have been two days old and obviously the growth of *Haematococcus* was a very young one, consisting of few individuals. At first sight the alga was taken for *Haematococcus droebakensis* since the most obviously distinctive feature was the presence of two pyrenoids. Closer examination however revealed the very different form of the anterior apex with its flat, widely separated papillae and characteristic insertion of the flagella, the angle between the flagellary tubes being obtuse, even wider than that of *H. lacustris*. It is on these characters that the specific distinction is based. The few individuals seen were fairly large and distinguished by the presence of numerous granules in the region surrounding the nucleus, a feature of motile cells recently formed from germinating zygospores. Unfortunately they were too few in number to indicate the range in size or possible variations in form, and a second visit to Zimbabwe planned for the following month was prevented by unprecedented rains.

Visits to the granite hill of Dombashawa in December, to several granite outcrops between the London Garage and the Bonda Mission in the Inyanga district, and later (January 1958) to a number of granite koppies 25 miles from Salisbury along the Shamva Road on the Gorhwe Native Reserve yielded no more material of this interesting species. There were plenty of pools, in nearly all of which the characteristic rotifer fauna, including both the species found at Zimbabwe, was present. In the first case, the pools were very young, in the others too old, and in none was *Haematococcus* of any kind collected. On the return journey from Rhodesia several pools on flattish granite outcrops near Palapye Road and Gaberones were examined, but without result so far as *Haematococcus* was concerned—in these cases again the pools were old. There can however be little doubt that, if the right times for the pools in question were found, *Haematococcus* would be present, and most probably *H. zimbabwiensis* will eventually be found to be widespread in Southern Rhodesia and the neighbouring territories.

IV. *HAEMATOCOCCUS LACUSTRIS* (Girod.) Rostafinski (1875); *H. PLUVIALIS* Flotow

Figure 6; Plate I, A

This cosmopolitan species is widely distributed in southern Africa in both artificial and natural habitats. Among the former the commonest are stone or cement bird-baths, for example, in gardens at the Cape; 'wild' localities include nearly all the localities in which *H. capensis* has been found in addition to others in which the latter species has not yet been collected.

The following records, while not exhaustive, will serve to indicate the ubiquitous nature of this species:

(*a*) *Artificial habitats*

Cement bird-baths in garden, Rusdon, Rondebosch.

University of Cape Town: appeared in cultures in the laboratory, e.g. culture of *Hydrodictyon africanum* (Miss E. L. Stephens); *Rhopalota* culture (Prof. Adamson); many algal cultures.

Wellington: Huguenot College Botany Laboratory, in *Achlya* culture, originating from the Stellenbosch Flats.

Grahamstown: rain water from the roof of the Botany Department, collected at the downwater pipe, and kept in the laboratory in beakers, turned pale red after a few days from the growth of *H. lacustris*; culture solution was then added resulting in the disappearance of most of the haematochrome and the culture becoming green. In succeeding years many liquid cultures, algal and otherwise, were contaminated;

→

FIGURE 6. *Haematococcus lacustris*.

A–C, mature motile cells: A, from a young growth, protoplasmic strands fine; B from an older growth, protoplasmic strands coarser and with inclusions, a single apical vacuole showing; C, polar view, strands intermediate between those of A and B; in all, protoplast vacuolated, some of the body vacuoles with granular or globular inclusions; sheath of haematochrome round the nucleus indicated by heavy shading.

D–M, asexual reproduction: D–J and L showing variations in the inclination of the plane of first division; D, change in shape preparatory to first division; E, F, first division complete; G, reorientation of the two resultant parts of the protoplast preparatory to second division, nuclei close to the surface, haemotochrome envelope confined to the inner surfaces of the nuclei; H–K, cells in which only one division has taken place, resulting in two zooids only, cell motile throughout, except in K; H and J, showing apices of the zooids not in the vertical plane, indicating a shift in the parts of the daughter protoplasts prior to differentiation, H showing pyrenoids, J none; K, exceptional case, cell immobile before escape of zooids which has been delayed; note membrane well separated from the protoplast and central position of nuclei, both features indicative of maturity of the zooids; L, M, motile cells producing four daughters, in L division has just been completed, in M differentiation of the zooids well advanced, zooids separating; apical zooid with two sets of flagellary apparatus including two eyespots is beginning to oscillate preparatory to freeing itself, much haematochrome present masking the parts of the zooids.

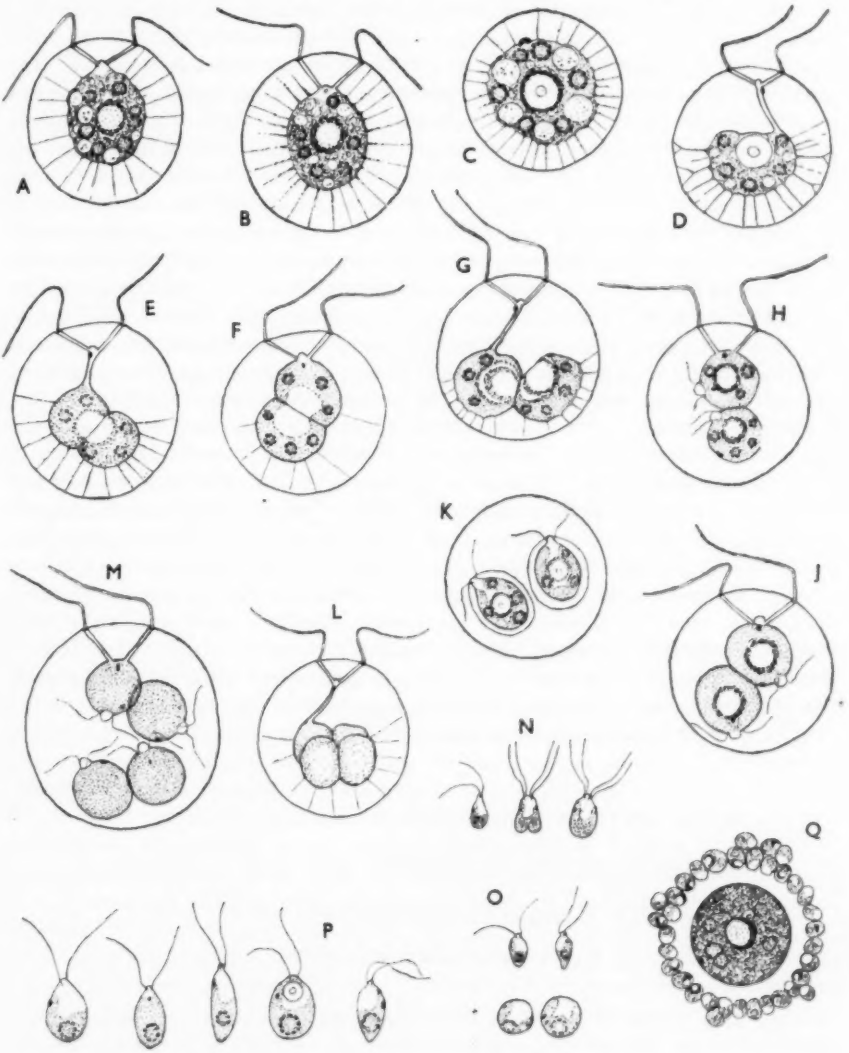
N–Q, formation of naked swarmers: N, gamete, conjugation and planozygote from rain-water pool in bed of the Palmiet River, the day after rain; no pyrenoids distinguishable but much stroma starch present; O, gametes and two zygotes which have come to rest, from a Signal Hill culture; P, larger type of swarmers showing varying shapes, no signs of conjugation and no zygotes seen, Signal Hill culture; Q, large motile cell which has come to rest and rounded off on agar, swarmers from Signal Hill and Rooi Krantz cultures coming to rest round it, kept from close contact by mucilaginous zone of wall, those from Rooi Krantz distinguished by presence of haematochrome which is beginning to accumulate round the apical nucleus, none in those from Signal Hill.

A–M ×500; N–Q ×1,000.

in fact, once the species has been introduced it is very difficult to prevent its entry into other cultures which may be in progress in the laboratory.

(*b*) *Natural habitats*

Western Province: In 'dassie reservoirs' in Table Mountain quartzite: koppie above Buffels Bay; on Table Mountain, Kalk Bay and Muizenberg mountains; near



Ceres, pools on rock at the top of Michell's Pass. Pools on granite: Mamre, Paarl Rock, and on rocks of the Malmesbury Series between Darling and Yserfontein. *Eastern Province:* Grahamstown, pools in quartzite of the Witteberg Series: mountain path above Somerset Street and at Signal Hill. Pools on hard shales of the Karoo Series: above pan 6½ miles out along the Cradock Road and in bed of the Palmiet River half a mile further along the same road, on Table Hill Farm. Rooikrantz (T.M.S.), upper Swartkops River, Uitenhage mountains (A. R. A. Noel). Amatola Mountains, pools in hollows of dolerite sill at Rockford, near the Hog's Back.

Haematococcus lacustris differs from all the other species in (*a*) structure: the protoplast is not drawn out into thick protoplasmic processes; instead it is connected directly with the outer membrane by numerous fine colourless threadlike strands which, especially in young growths, are sometimes so thin and delicate that it is only with difficulty that they can be distinguished. At other times they are much thicker, contain numerous vacuoles and granules and are obvious even under low powers of the microscope (fig. 6, B, C). Occasionally the protoplast is drawn out very slightly at the point of attachment of the threads (fig. 6, B) suggesting the possible mode of origin of the thick protoplasmic protusions found in the other species. The chloroplast is clearly parietal, lining all but the extreme apex of the protoplast and containing numerous pyrenoids. The stigma is normally small and inconspicuous, often masked by the abundant haematochrome, and the cells are not markedly phototactic, but this character is subject to considerable variation. The anterior pole is widely distant from the outer membrane with which it is connected by two long flagellary tubes diverging at approximately a right angle from one another—much more obvious than the far shorter tubes present in *H. buetschlii*, *H. droebakensis* and *H. capensis*. Only in *H. zimbabwiensis* do the flagellary tubes diverge more widely.

And (*b*) in its life history: firstly in the ability of the motile cells to come to rest, secrete a wall and form resting cells. If the wall of such a cell remains thin, it may continue to grow, reaching a much larger size than the motile cell, or it may become very thick, resulting in a resistant hypnospore. Secondly, and probably in consequence of this ability, asexually formed resting spores have to a large extent replaced the sexually produced zygospore as the resistant resting spore which tides the organism over adverse periods. Consequently sexual reproduction is of very rare occurrence.

These characters are discussed more fully later.

PART II

STRUCTURE, DEVELOPMENT AND LIFE HISTORY

I. *HAEMATOCOCCUS CAPENSIS*

1. Asexual Reproduction

(*a*) *Division*

The average size of the mature individual of *H. capensis* var. *capensis* is approximately $44 \times 49\mu$, i.e. the membrane is almost spherical, with a protoplast $22 \times 33\mu$.

But the size in a vigorous growth is variable; much smaller cells may divide and quite often larger cells occur. Examples of the latter are as follows: cell $53 \times 58\mu$, with protoplast $24 \times 40\mu$; cell $58 \times 65\mu$, protoplast $27 \times 53\mu$; cell $62 \times 66\mu$, protoplast $22 \times 53\mu$. The size at maturity varies considerably; rather curiously, although the Bonteberg material was highly vacuolated, the cells were among the largest ever seen. In general, var. *piriformis* is smaller than var. *capensis*, while var. *torpedo* may attain a greater length, but is typically narrower, the protoplast almost reaching the flattened sides of the membrane.

The first noticeable signs of preparation for division are changes in the shape of the protoplast. The anterior apex becomes elongated and drawn-out, the bulk of the protoplast lying in the posterior two-thirds of the cell (figs. 1, G; 3, D). Meanwhile the stigma has begun to move towards the anterior pole until eventually it may lie just behind the insertion of the flagellary tubes or at other times lower down in the apical prolongation of the protoplast (Pl. II, D). Withdrawal of the protoplasmic processes begins in the anterior part of the cell, those towards the back usually persisting till later. Since movement of the cell continues actively throughout the whole process—and this applies to all species of Haematococcus—it is obvious that the plane of first division cannot coincide exactly with the polar axis of the cell; instead it is inclined to the polar diameter, the angle of inclination varying considerably in the individual cell. As a consequence, the change in the shape of the protoplast is accompanied by a rearrangement of its parts—the chloroplast slews round so that the colourless zone comes to lie somewhat to the side, this zone marking the position of the anterior pole of the protoplast. Examination with the $\frac{1}{12}$ in. oil immersion lens shows the nucleus lying in this zone near the surface of the protoplast. The pyrenoid does not divide; it may disappear early in the process of division or may persist till a later stage. As Fritsch points out (1935, p. 93), the first division is therefore always technically longitudinal since it is longitudinal to the rearranged protoplast even if inclined, no matter at how great an angle, to the longitudinal axis of the cell as a whole (figs. 1, G, H; 3, D–F).

In some cases no further divisions follow and only two daughters result. More usually a second division, likewise longitudinal, in a plane perpendicular to the first, follows and four daughter cells are formed. In asexual reproduction in the majority of cases no further divisions take place but sometimes in particularly vigorous growths a third or even a fourth division may result in the formation of eight or, more rarely, sixteen daughter cells, as for instance in the Bonteberg material. In general, successive divisions are practically simultaneous and the resultant cells all equal in size, but occasionally, particularly in old cultures, irregularities in division due mainly to unequal cleavage may occur, some only of the daughter cells dividing again so that five, six or seven daughter cells may be produced. By far the commonest number however is four.

The nucleated apices of the resultant cells are always directed inwards as in *Volvox*, each cleavage being followed by some degree of rearrangement within each segment (figs. 1, J; 3, G). Once division is complete, the long-drawn-out apex gradually contracts again until the anterior daughter cell lies close to the membrane,

with the conspicuous parental stigma usually near the anterior pole. Some of the posterior protoplasmic processes may survive cell division; if so, they too are now withdrawn and the divided protoplast is connected with the outer membrane only at the anterior pole by the flagellary apparatus (figs. 1, L; 3, H–K).

(*b*) *Subsequent development of the daughter cells*

Completion of division is followed by a period of maturation ending in the escape of the zooids* formed by the daughter cells. This maturation involves change in shape accompanying the gradual separation of the segments, reorganization of their protoplasts, formation of the flagellary apparatus and stigma, secretion of a cellulose membrane and separation of membrane and protoplast. The maturation period is comparatively short, some one to three hours or so, the movement of the parent cell continuing throughout. Apparently this movement tends to slow down during actual nuclear division, speeding up once more as soon as each division is complete. Changes in the shape of the daughter cells soon become apparent, progressing as cleavage and subsequent separation are completed; the more or less rounded segments become elongated with definite anterior and posterior poles, the nucleus lying in the colourless anterior apex. In some cases both are pointed, in others the posterior pole is more rounded depending to some extent on the variety or form in question. As separation begins a faint flickering becomes noticeable at the nucleated apex of each zooid, marking the beginning of emission of the flagella. This gradually strengthens until the flagella may be clearly seen, reaching out almost to the parental membrane. More or less simultaneously with this the cellulose wall laid down on the surface of each little protoplast becomes apparent and the embryo zooids, now separated from one another, begin to move about within the parental membrane. This movement, at first feeble, gradually strengthens as the flagella elongate, and though never as rapid as in water, serves to demonstrate the watery nature of the contents of the cell membrane. Since the cell remains motile throughout the whole process, it follows that the parental flagellary apparatus must remain operative; the anterior zooid, therefore, though freed from its sister zooids, remains attached to and activating the parental flagellary apparatus, but at the same time it is maturing just as are its sister cells. Its posterior pole, the anterior pole of the parent protoplast, bears the flagella and contains the large stigma of the parent cell, while at the same time at the opposite apex, its anterior pole, its own flagella and stigma are developing, its shape changing and its protoplast becoming reorganized. For a time, therefore, it has as it were two brains at opposite poles, working independently of one another but controlled by the same nucleus (figs. 1, L; 3, H–M). This remarkable phenomenon was first noted and figured by Blochman in *Haematococcus buetschlii* (pl. 1, 9) and subsequently by Wollenweber (1908, pp. 260–1, T. XIV, 8) in *H. droebakensis*. It is also found in *H. lacustris*.

Separation of the membrane from the protoplast usually begins during the movement of the zooids within the parent vesicle although it is probably not fully completed till after liberation. The actual process has been watched in *H. capensis* var. *torpedo*

* The term *zooid* is convenient to denote a motile cell formed in asexual reproduction, as distinguished from a *zoospore*, which is the motile cell formed on germination of the zygospore.

in which the inner clear zone of the wall reaches its greatest development at the posterior pole. A slight convulsive movement was seen, possibly due to an expulsion of liquid resulting in a shrinkage of the protoplast with a corresponding stretching of the membrane; as a result a space between membrane and protoplast now became visible (fig. 3, L). At this stage it was not possible to make out the protoplasmic processes, but they are probably present from the beginning as delicate colourless strands traversing the developing space between protoplast and membrane, the more massive protuberances of the protoplast developing later. They may however become visible at an early stage at the posterior pole (fig. 3, M).

As a rule the parental pyrenoid has disappeared during the process of division; as the zooids develop, at first stroma starch is present throughout the chloroplast, but as they mature, a single pyrenoid appears slightly posterior to the centre of each little cell.

(*c*) *Escape of the zooids*

Movement of the three posterior zooids within the vesicle gradually strengthens; finally, probably owing to increasing osmotic pressure resulting from the activity of enzymes secreted by the zooids, the membrane ruptures and as it does so, first one, then the other two posterior zooids swim out into the water. Movement of the whole cell has grown slower and now the anterior zooid can be seen oscillating back and forth with a wriggling movement under the action of its own elongating flagella while still continuing to work the parental flagella, but more and more feebly. Finally the little cell succeeds in breaking loose from the parental flagellary tubes, movement of the parent cell immediately ceases completely, and the last daughter zooid follows its sisters out of the vesicle into the surrounding water. In the first stages of its career as an independent organism this cell still possesses two eyespots, the large parental eyespot near its posterior pole and its own, much smaller one, near its anterior apex. Soon, however, the former begins to disintegrate and it is left with its own small stigma. The stigmas in young daughter cells are usually close to the anterior apex, but later assume a more or less equatorial position until the time for division approaches.

When first liberated, the zooids are elongated, either pear- or spindle-shaped. Soon separation of the membrane, begun while still within the parent cell, is completed; as the space between membrane and protoplast widens, the delicate protoplasmic strands, firmly attached to the membrane by their distal ends, draw out the surface of the protoplast to form the thicker proximal ends of the processes into which the chloroplast extends, and the zooid gradually assumes the form typical of the species or variety (fig. 3, J–M).

Slight variations may occur in the different forms such as the very noticeable separation of wall and protoplast at the posterior pole referred to above, and the marked extension of the protoplasmic processes into the posterior polar extension found in the varieties *piriformis* (particularly its form *caudata*) and *torpedo*. The general sequence of events throughout, however, is as described here. Careful watching with the oil immersion lens is needed to elucidate fully the details of development. With

growth, the pyrenoid increases in size, the eyespot usually moves nearer to the equatorial plane, while the nucleus, at first apical, assumes a nearly central position, just anterior to the pyrenoid.

2. Sexual Reproduction

(*a*) *Division*

In general, sexual cells are rather smaller than those reproducing asexually (cf. fig. 3). The first two divisions preparatory to the formation of gametes are like those in asexual division, but here further divisions, at least two, more often four to six, follow in close succession, each taking about half an hour to complete. The mode of division is essentially similar to that in *Volvox*, such as the division of the male initial cell to form spermatozoid globoids in the *globator* section, but still more closely comparable with the formation of the germ colony—successive planes of division are more and more inclined inwards, always accompanied by slight divergence of the nucleated apices of the resultant cells (Pocock, 1933, figs. 5, 6, 9). Consequently, at first a hollow bowl, and eventually a hollow spheroid of small cells results, the nucleated apices of which are always directed inwards (fig. 3, O, Q; Pl. II, D–G), while this spheroid remains open by a well-defined pore the position of which is primarily determined by the plane of the first division. If this was closely inclined to the longitudinal axis of the cell, the pore lies close to the anterior pole (Pl. II, D); if the angle of inclination was greater, the pore lies more or less laterally, while sometimes the plane of first division was practically transverse to the long axis of the cell and the pore is almost equatorial. Apparently any of these are possible, but in *H. capensis* it is more usual for the pore to be close to the apex, i.e. for the angle of inclination of the plane of the first division to be small (fig. 3, D, F, O). Very occasionally as few as sixteen cells were seen in small but fully mature sexual cells and still more rarely only eight have been seen; in normal vigorous cells, however, the usual number is 128 or 256, while occasionally as many as 512 may be formed, i.e. normally five or six divisions succeed the two initial divisions, the number of cells formed depending directly on the size of the sexual cell prior to the commencement of division. Here again, in normal growths division is very regular, all parts dividing practically simultaneously (figs. 2, D, C; fig. 3, N–Q). Irregularities in division are rare and characteristic of old or otherwise abnormal cultures. In such cases the resultant gametes vary in size and it is doubtful whether all are viable.

(*b*) *Maturation*

Completion of division is normally achieved by the evening or the early hours of the night. It is followed by a comparatively lengthy period of maturation during which the cells continue actively motile. This may last for some hours so that in active cultures by midnight the population consists of undivided motile unicells among which move innumerable sexual individuals, both showing some variation in size, but all the latter containing fully formed globoids of developing gametes. The constituent cells of these globoids are small, typically very uniform in size and more or less rounded (fig. 2, D, C; Pl. II, F, G). When mature, each gamete bears two

flagella arising from the inwardly directed nucleated apex, exactly as in *Volvox* globoids and germ colonies.

(*c*) *Escape of the gametes*

Escape has been observed only at night, occasionally as early as 9 p.m., but usually from 11 p.m. onwards, becoming increasingly abundant until a peak is reached in the early hours of the morning, when the population includes innumerable minute gametes dashing hither and thither among the vegetative and remaining sexual cells.

The whole process of liberation has been watched repeatedly and shows the following sequence of events, attention being concentrated on a cell containing a large mature globoid:—

1. In the fully mature sexual cell, actively motile but with a steadier, more stately movement than in the vegetative motile cell, the cells of the globoid appear more or less rounded and very closely compacted (fig. 2, D).

2. Movement begins to slow down and a change in the appearance of the globoid begins to appear, the constituent cells appearing slightly less closely packed.

3. A slight movement, hard to define and best described as a kind of faint shudder, passing over the whole globoid, can sometimes be seen.

4. The pore of the globoid is seen to be enlarging and the whole appearance suggests preparation for inversion as seen in *Volvox* (fig. 2, E, F; Pl. II, F, G).

5. Instead of inversion following, however, the constituent cells begin to separate; strands connecting the cells and the flagella can sometimes be distinguished as separation begins (fig. 2, G, H).

6. At this stage the cells are more or less isodiametric with lengthening flagella, but as development proceeds they become elongated and spindle-shaped (fig. 2, E, F; fig. 3, Q).

7. Suddenly the whole mass of cells disintegrates into a crowd of minute swarmers moving with great rapidity within the parental membrane, one or sometimes two or three of the tiny cells remaining attached to the parental flagella (fig. 2, J). As movement quickens the elongation already begun becomes more marked.

8. After a few seconds of this intense activity, the membrane is ruptured and the gametes stream out into the water.

9. With the separating of the gametes movement of the parent cell, which had begun to slow down during the preparatory stages of breaking-up of the globoid, finally ceases, except that in some cases a feeble rocking movement showed that the tiny anterior cell, still attached to the flagellary apparatus, was still activating it, though too small to cause locomotion. Soon this too ceased and the remaining daughter cell, usually containing the parental stigma, disintegrated.

The process of liberation of the gametes is achieved with extraordinary rapidity. After the preliminary 'shudder' which heralded the break-up of the globoid the whole process—changes in shape of the cells, emission of flagella, separation, swarming within the parent vesicle, rupture thereof and final escape—is such a swift progression

that words are inadequate to describe it in detail; further, unless a number of cells in various stages are killed with iodine or otherwise fixed and stained and then examined with the oil immersion lens it is impossible to distinguish certain details, as for instance the developing flagella and the connecting strands.

(d) Conjugation

The liberated gametes are more or less spindle-shaped, with comparatively long flagella (more than body length), a conspicuous eyespot and a pale green chloroplast lining the posterior part of the cell, rather to one side, in which a small pyrenoid can usually be distinguished (fig. 2, K).

Liberation is quickly followed by 'clumping' leading to conjugation and since many individuals are liberating gametes simultaneously the chance of gametes from different individuals fusing is high, but so far as could be ascertained, gametes from the same parent could also unite.

The gametes are small, $3 \times 5\mu$ to $4 \times 6\mu$ as a rule, but since they are highly metabolic, shape and proportion of length to breadth are so variable that accurate measurement is difficult. In most cases conjugating gametes were of equal size but occasionally a slight but appreciable difference in size was noted (figs. 2, K, L); despite this, there seems to be true isogamy; whether there is heterothallism or not has yet to be determined.

Contact is made laterally, the apices uniting first; union continues towards the posterior pole until it is completed. The beginning of conjugation is usually marked by a rapid spinning movement, either on one spot or accompanied by progression through the water. On completion of union the movement changes completely; the quadriflagellate planozygote ($4 \times 9\mu$ approximately) swims with a rather steady movement, continuing actively motile for some time. Fairly soon, however, it comes to rest, the flagella attaching themselves to some substratum, rounds off and secretes a wall to become the resting zygospore, 5 to 7μ in diameter (fig. 2, M).

When sexual individuals were mounted in a hanging drop, liberation and conjugation proceeded vigorously; as the resultant planozygotes began to come to rest, they collected round the edge of the drop, chiefly on the side away from the light, and there rounded off. The following morning no gametes remained but the drop was surrounded by a ring of zygotes. Sexual reproduction proceeds well in agar plate cultures (Pl. II, B), and for many purposes such cultures are preferable to those in liquid media, particularly for the study of the further development of the zygote.

In the planozygote and newly formed zygospore two stigmata and in some cases two pyrenoids, in others one only, can be seen (fig 2, M). The eyespots persist for a time after the secretion of the wall, then disintegrate. On agar, enlargement of the zygospore usually follows (Pl. II, H, K), but in nature the zygospore usually remains small and thickening of the smooth wall immediately follows. As the pool dries up the chlorophyll is replaced by haematochrome of a yellowish-red tinge, a very different colour from that characteristic of *Haematococcus lacustris* so that it is possible to distinguish the zygospores of *H. capensis* from resting spores of *H. lacustris*.

(*e*) *Development of the zygospore*

Zygospores were obtained and matured in both liquid and agar plate cultures and both have been germinated, but the latter method is preferable for watching the later stages of development. According to Wollenweber, the use of an agar substrate predisposes *Haematococcus droebakensis* to sexual reproduction and in the case of *H. capensis* the agar plate method (0·75 per cent agar made up in Juller's algal culture solution) was found to be very useful.

The photographs reproduced in Plate II, A, B (unfortunately fogged) show two single-cell inoculations of *H. capensis* var. *torpedo*; A shows a group in which four successive divisions have resulted in the formation of sixteen normal cells which on addition of a drop of liquid would at once become motile. The second photograph, B, shows another one-celled inoculation in which division has been carried further but less regularly; eighteen of the progeny are reproducing sexually, two still in early stages of division; other cells are normal motile cells, the smaller ones of which have evidently been formed recently, while two have rounded off to form resting cells.

Since the agar used is very dilute, the weight of the cells, small though it be, is sufficient to depress the surface of the agar slightly so that each is surrounded by a tiny well of liquid in which the gametes may swarm. If liberation is delayed, addition of a small drop of liquid, whether culture solution or distilled water, precipitates liberation and subsequent conjugation proceeds actively. Again the planozygotes tend to collect round the edge of the drop where they round off to form tiny zygospores (usually 5–7μ in diameter) though sometimes larger ones occur. Both planozygote and newly formed resting spore show two eyespots. As the wall thickens these disappear and usually only one pyrenoid persists even if two were present to begin with (fig. 2, L, M). Mature zygospores with more than one pyrenoid may occur but are usually rare (Pl. II, K).

(*f*) *Maturation of the zygospore*

In nature it is probable that the zygospores remain small until immediately prior to germination; small size would make for more effective protection during periods of drought, since, closely adpressed to the soil particles, the hygroscopic water of the soil would serve to keep the tiny zygospores moist where it would not be sufficient in the case of larger spores.

Kept on agar, however, wall formation is soon followed by a period of enlargement which ceases if the agar begins to dry out. The colour, at first very pale green, becomes darker green, then begins to change owing to the replacement of chlorophyll by haematochrome, at first a pale golden colour or orange-red very different from the bright scarlet-red of *Haematococcus lacustris*; later as the wall thickens the colour darkens to a deep reddish brown. The spores show great variation both in colour and size—while still greenish-gold in colour the diameter may vary from 4μ to 27μ. As older spores enlarge and become deep golden-brown, diameters of 49–58μ and even 62μ were noted. Although no doubt the size to a great extent indicates differences in age, other factors help to determine relative size. These are partly external, e.g.

degree of crowding, but partly also some inherent tendency in individual spores which determines when enlargement shall take place and to what extent.

In the resting spore, or more correctly in the vegetative state of the zygospore during which, though not active, there is nevertheless continued growth, the nucleus is central and there is a single large pyrenoid, fairly easily distinguished in the young spore but often obscured in the older darker-coloured stages. In agar plate cultures, the wall does not become very thick; possibly in natural dryer conditions it may be considerably thicker.

(*g*) *Germination of the zygospore*

After a period of growth, the addition of a drop of culture solution will usually bring about germination, provided the drying-up of the agar has not proceeded too far. Alternatively, especially in the latter case, the zygospores (Pl. II, H, K, M) may be gently detached from the agar by using a small sable paint-brush and the liquid containing the freed spores transferred to a watch glass, where the further development can be easily watched. In general, to initiate germination, 50 per cent culture solution proved more effective than full strength. In older cells pretreatment with glass-distilled water for a few hours is sometimes necessary. Transference to culture solution may then start germination.

The first visible sign of germination is a change in shape of the spore which ceases to be spherical, elongating slightly in one direction, which now becomes the polar diameter. At the anterior pole a slight thickening of the inner layer of the wall initiates the protrusion of this pole which heralds the formation of the vesicle into which the expanding protoplast protrudes, at first completely filling the vesicle as it forms.

The protoplast in this protruding pole appears less dense than the rest and with the oil- or water-immersion lens an optical section shows a clear anterior portion into which the nucleus has moved from its central position (fig. 2, O, P). It seems certain that it is at this stage that meiosis takes place. Direct observation of living spores has shown not only the presence of the spore nucleus (diploid) in the clear apex of the protoplast but at a later stage first two and then four nuclei (fig. 2, Q, R), presumably haploid. In a few germinating spores meiosis has in fact been demonstrated cytologically at this stage (Cave and Pocock, unpublished).

(*h*) *Formation of the zoospores*

Once nuclear division is complete, the nuclei resume the central position and cleavage of the cytoplasm follows. The plane of the first division is slightly oblique to the longitudinal axis and is followed by a partial rotation of the two resultant protoplasts so that the second cleavage divides the protoplast more or less tetrahedrally. In small and average-sized spores cleavage is now complete, resulting in the formation of four zoospores (fig. 2, S, T), but in spores which have enlarged considerably before germination, the first two divisions may be followed by a third, the nucleus in each part dividing mitotically; cleavage follows and eight zoospores result. Occasionally the two primary cleavages are not exactly equal; in such cases

only the larger part or parts may divide and as a result, other numbers of zoospores may be formed—5, 6 or 7 have been noted. The size of the segments is obviously the deciding factor in determining the number of cleavages; apparently there is a definite limit to the size of a zoospore (Pl. II, J–M).

(*i*) *Escape of the zoospores*

The protoplast as a whole passes gradually into the enlarging vesicle which at first it nearly fills, its parts adhering closely to one another (fig. 2, S); as division and subsequent organizing of the zoospores near completion enlargement of the vesicle continues towards the anterior pole into which the protoplast presses, while at the posterior pole a space gradually forms (fig. 2, S, T). Sometimes the zoospores separate and begin to move within the vesicle; more usually they remain in contact until a later stage. Eventually the vesicle is ruptured at the thin distal end and the zoospores escape; if they are already moving within the vesicle they swim out one by one, otherwise they are shot out in a single mass by the collapsing of the vesicle as the wall ruptures. If the zoospores are shot out, there is a brief quiescent pause as they reach the water. This lasts only a few seconds after which the zoospores swim away (Pl. II, J, K, M).

To begin with the zoospore is elongated-ovoid (pl. II, L), very dense golden-brown in colour, with a clear colourless apex in which the nucleus lies, bearing the rather short flagella (less than body length). A separate membrane has never been observed in the zoospore before escape, but it develops soon after liberation and the zoospore soon assumes the characteristic form of the typical motile cell. The pyrenoid and eyespot become visible as the abundant haematochrome is replaced by chlorophyll and the nucleus takes up a nearly central position in the cell. Small droplets of haematochrome of the colour characteristic of the species may survive for some time in the protoplast of the young cell, but this soon disappears entirely. Development is rapid and at a quite early stage the only sign that a motile cell has developed from a zoospore is the occasional presence of droplets of haematochrome.

3. Diurnal Rhythm

If a natural growth or a normal healthy culture of *Haematococcus capensis* be watched throughout the twenty-four hours a well-defined rhythm becomes apparent. The original *Haematococcus* pan at De Klip was comparatively easy of access, very constant in its formation whenever it rained and in its production of an almost pure growth of the alga. It was therefore ideal for study and was visited at many different times of day at each of which samples were collected. These were examined at once and kept under observation for study at leisure throughout the day and night. The natural water was usually supplemented by addition of small quantities of culture solution.

In the early morning the algal population consisted of actively motile cells of varying sizes—the larger being those which had nearly reached maturity the previous evening but had not yet begun to divide, the smaller and more numerous the progeny of asexual reproduction during the previous afternoon. Usually there was no sign of

sexual reproduction. With morning light growth had been resumed, increase in size was rapid and soon the early period of growth was followed by one of active asexual reproduction, the onset of which was marked by the increasing number of larger cells showing preparation for division. By mid-afternoon most of these larger cells were liberating daughter cells or dividing, and the population was again a mixed one with many small, newly formed cells among the large undivided or dividing individuals.

Now however a number of the dividing cells were obviously sexual and by evening the stage of asexual reproduction had been largely replaced by one of sexual reproduction—here and there cells producing daughter cells might still be seen, but the great majority of those dividing were forming gametes. Liberation of the latter apparently always takes place at night; during the early hours maturation of the gametes is in progress, escape not beginning till about 9 p.m. and increasing rapidly until a peak period is reached from about midnight to 2 a.m., after which the number of gametes decreases rapidly and, as conjugation is achieved, the planozygotes soon cease moving, settle down to form zygospores and all sign of swarmers among the vegetative cells entirely disappears.

This rhythm—successive periods of growth, asexual reproduction, sexual reproduction, culminating in the formation of zygospores—has been observed repeatedly in both natural growths and cultures. Variation in actual times of course is frequent, depending on external conditions, and there is considerable overlap, but in general the sequence is clear. Variations in temperature, nutrition, amount of sunshine, rainfall or other precipitation, all modify considerably the incidence and duration of each period.

The fate of the zygotes is not easily studied directly either in the field or in gross culture, but the use of hanging drop—used already by Blochmann—and agar plate-cultures makes it possible to complete the study of the behaviour and development of the gametes, planozygotes and zygospores and thus to supplement observations in the field and confirm the deductions made therefrom. The most striking feature is the rapidity with which the alga appears after rain and passes through all its stages, thereafter in most cases disappearing completely from the pool in which it had abounded, often even before the latter has dried up. More usually, its duration is determined by that of the pool; but even though the period during which the pool is dry may be very brief, a fresh rain will usually result in a new growth of the alga.

The different varieties and forms all fall into the same general plan, showing only minor variations from the account given above; the study was made chiefly on *Haematococcus capensis* var. *capensis* from De Klip, and, to a somewhat lesser degree, from the Bonteberg, but other varieties, particularly var. *torpedo*, have also been studied extensively.

II. *HAEMATOCOCCUS DROEBAKENSIS* Wollenweber

This species has been so carefully studied and described by Wollenweber (1908) that here it is only necessary to note any special features which appeared in the Stonehenge material.

The original samples consisted mainly of nearly globose individuals (fig. 5, A, C)

similar to those figured by Wollenweber as figures 1*c* and 6 on Plate XII, but in the later cultures this type of cell was rare and far the commonest form was the elongated sub-cylindrical type (fig. 5, B, D) which, judging from Wollenweber's figures, was also the case in his material. Such cells were cometimes fastigate with pointed posterior pole, or more often had rounded posterior poles without an apiculus.

An outstanding feature of the South African cultures was the tendency to multiplication of the pyrenoids as the cells matured, but always with retention of two distinct groups, one anterior, the other posterior to the central vacuole (fig. 5, B, D). The increase in number was characteristic of large fully developed cells in well-nourished cultures and was not a constant feature, nor apparently a provision for the pyrenoids in daughter cells. The primary pyrenoids could usually be distinguished by their larger size (fig. 5, C). The nucleus usually lay rather to one side of the central vacuole, sometimes towards the front, more often towards the back. The structure of the chloroplast, with massive portions in the two poles connected by a very thin girdle of pigmented protoplasm surrounding the vacuole, is characteristic of all three two-pyrenoid species.

As might be expected from the construction of the cell, the first plane of cleavage is usually more widely inclined to the longitudinal axis of the cell than in *H. capensis*, but this is by no means always the case (cf. fig. 5, E–H). In all species this appears to be a very variable feature, cf. Wollenweber Tab. XIII, fig. 3, Tab. XIV, 2*b*, etc., also Blochmann in *H. buetschlii* as well as in *H. lacustris* (see below).

In sexual reproduction the number of gametes is usually smaller than in *H. capensis* (fig. 5, H); occasionally a slight difference in size was noted in the gametes of a conjugating pair but this was not sufficiently constant to be regarded as anisogamy. In the later cultures, although sexual cells appeared regularly, zygotes were seldom formed and after a few years the cultures were finally lost. This suggests the possibility of the presence of heterothallism in the Stonehenge strain.

III. *HAEMATOCOCCUS LACUSTRIS*

Probably no single species of alga has given rise to more discussion and controversy than this ubiquitous 'rain alga', nor had more papers written about it. But despite its wide distribution and the extensive literature dealing with it, there still remains much uncertainty as to certain details in its life cycle and behaviour. The development of a very efficient system of asexual reproduction admirably suited to the peculiar habitat has resulted, as is often the case, in the replacement to a very marked degree of asexual for sexual reproduction. Thus the life cycle is by no means the clear-cut story characterizing the other species of *Haematococcus* (*H. zimbabwiensis* always excepted in the present state of lack of knowledge of this species). Its very commonness has led to some neglect in recent years, and curious misconceptions have crept into the recent literature.

1. The Motile Cell

In this connection it is necessary to emphasize the fact, that as in the other species, the normal form is the *motile* cell; it is through the activity of the motile cells that the

population is primarily built up. In nature and in cultures which approximate to normal conditions the motile cells formed on germination of resting cells rapidly increase in size, divide while still motile to form typically two or four daughter cells which, as pointed out by Blochmann, become motile within the parent membrane and finally escape, the parent cell only becoming immobile as this occurs. In the process the unique phenomenon of 'two-headedness' in the anterior daughter, first recorded by Blochmann in *H. buetschlii*, occurs also in *H. lacustri*.

During division of the motile cell, the rearrangement of the organelles both in the parent protoplast and in the daughters as cleavage takes place is very noticeable, more so than in any other species since the nucleus is usually more or less surrounded by haematochrome, and as the position of the nucleus changes the rede nvelope surrounding it moves with it. Thus when, prior to division, the nucleus moves to the surface of the protoplast, the red sheath also changes its position. Further, cytoplasmic cleavage may begin before nuclear division is complete and often after the first division the two resulting segments may partially separate and their contents reorientate themselves independently of one another. Very often only two daughter cells are formed instead of four, the more usual number in *H. capensis*. The daughter cells are very characteristic—short and broad with a colourless dome-shaped apical papilla, very conspicuous against the usual dark red colour of the cell and particularly so where the daughter cells are already motile, but even in the anterior daughter still attached to the parent this papilla is well developed (figs. 6, H, J, M). An interesting result of the reorientation within the segments is the fact that the papillate apex may be differentiated laterally on the segments (fig. 6, H, J).

2. The Non-motile State

Once the population has been built up by division of the motile cells, the formation of immobile cells becomes noticeable. Under certain conditions, the exact nature of which is still largely a matter of conjecture, the motile cells may pass into a resting state. The flagella are withdrawn and the protoplast rounds off within the membrane and secretes a wall. This may become thick and the cell pass at once into the resting condition, forming a resistant hypnospore, which at first may be red in the centre with a more or less extensive green outer zone. In fully green cultures the resting cells may at first be green throughout, but more usually even they have some red pigment. As a rule the hypnospores soon become wholly red, the colour darkening until it is so intense as to appear almost black. The changes in the pigment are accompanied by changes in the stored food, fats to some extent replacing the starch. Such dark red, thick-walled cells are highly resistant to desiccation.

Instead of passing at once into this resting state, however, the non-motile cells may remain comparatively thin-walled and pass into a condition of further development, the so-called palmelloid state, during which the size may increase enormously; or the contents may divide to form a number of smaller rounded cells and these in turn form quiescent cells which continue growth. The parental membrane may at first persist round the immobile cell, development taking place within it, but eventually it disappears. As the water of the pool dries up, these cells, too, develop thick walls

and become hypnospores, the walls becoming progressively thickened until the cells are most efficiently protected.

3. Germination of the Hypnospore

On germination, the behaviour of the nucleus of the hypnospore differs from that of the motile cell. In the latter, cytoplasmic cleavage either accompanies or follows directly on nuclear division. In the hypnospore, on the other hand, successive nuclear divisions follow one another rapidly and normally it is only after nuclear division is complete that cytoplasmic cleavage begins. It is quickly completed and each part may either round off immediately to form a resting cell once more or, if conditions are favourable, may develop into a normal motile cell, typically deep red in colour. In the latter case, changes in the wall resulting in the formation of a vesicle into which the protoplast extends are similar to those characterizing the germination of resting spores throughout the Volvocales, be they produced sexually or asexually. The number of zooids produced depends directly on the size of the hypnospore and, in the case of the enormous dark red spores which are sometimes seen, may be very great. The wall of the zooid is secreted before escape but separation of the membrane from the protoplast does not become apparent until later after the little cell has been motile for some time. Motile cells produced on germination of hypnospores are commonly very dark red.

Thus the non-motile cells of *H. lacustris* may function not only as hypnospores to tide the organism over periods of drought, but may also supplement the work of the motile cells in adding to the population.

The factors operative in bringing about the formation of non-motile cells and development of haematochrome include water supply, amount and nature of nutrient salts, particularly nitrogenous matter, illumination and oxygen supply. There is still much research needed before the exact chemical relationships are clarified. In nature, the nutrient medium is typically a very dilute one, the alga occurring in newly formed rain-water pools on rock, and there is usually considerable haematochrome present. As already described, addition of Juller's solution resulted in a more or less complete disappearanc of the red pigment, which reappeared again as the culture aged and the nutrient salts were becoming exhausted, particularly if the culture was exposed to strong light. On the other hand an abnormally strong nutrient medium may also result in a change from the motile to the non-motile state, particularly where organic matter is abundant.

4. Sexual Reproduction

In most groups of plants it often happens that where, in some species, vegetative reproduction is well developed, sexual reproduction becomes rarer. In *Haematococcus lacustris* so efficient has the method of non-sexual reproduction become and so well adapted is it to the environment, that sexuality has almost disappeared from the life cycle, and where it persists its appearance has been shifted from the motile phase, where as yet it is unknown. In 'domesticated' strains it has apparently entirely disappeared. Wollenweber (1908, p. 279) remarks 'as to gamogony in *H. pluvialis* it is

strange that it is constantly suspected but has never been established'. As early as 1844 Flotow observed naked swarmers to which subsequent workers variously gave the names microzoospores or microgonidia.

The gametic nature of the 'microgonidia' was finally demonstrated by Florence Peebles (1909). Schulze (1927), who gives a very complete bibliography, attempted to repeat Peebles's results, at first with no success but finally 'in March' he succeeded in obtaining gametes from various strains and watched conjugation in material both from Germany and from Russia. Unfortunately he gives no indication as to why his later attempts succeeded. Elliott (1934) obtained swarmers but failed to find any evidence of conjugation; he describes the naked swarmers as being '20μ or less' in length (p. 253), whereas Schulze states that they were never more than 9μ. Droop (1956, pp. 60, 62) obtained gametes in various races and found that copulation never occurred unless two clones of opposite mating types were cultured together. He describes the gametes as larger than those of *H. droebakensis* and different in shape, 'never attenuated or pointed behind'. Apparently he did not observe gamete-formation in nature. He states that gametes of *H. pluvialis* were shown to be facultative 'by the simple expedient of separating gametes in the early stages of copulation under the dissecting microscope and culturing them apart from one another' (p. 61), whereas gametes of the other two species (i.e. *H. droebakensis* and *Stephanosphaera pluvialis*) 'failed to grow when similarly isolated'. He gives no details as to how the material in question was treated to obtain gamete formation. Peebles never observed gamete formation in the field but obtained it readily from newly formed resting spores. She clearly describes the methods used to obtain gametes and emphasizes the need for close observation if conjugation is to be observed, since the period of copulation is very brief, occurring immediately after liberation of the gametes. Other workers however have recorded the appearance of swarmers (i.e. gametes; the sense in which Droop uses the term 'swarmer', i.e. for the typical motile *Haematococcus* cells, is misleading and surely not warranted by accepted usage) on the germination of old resting spores; Hazen (p. 224) obtained them from material which had been frozen for a great part of the winter, as well as from resting cells collected on the sides of the culture vessel.

On May 5, 1938, the day following rain, *Haematococcus lacustris* was collected in small rain-water pools formed in hollows of exposed rocks in the bed of the Palmiet River on Table Hill Farm, 7 miles from Grahamstown. This is a small intermittent river consisting of a succession of disconnected pools except immediately after heavy rain when it runs strongly for a short time. In one of the rock-pools the material consisted of numbers of orange-red motile cells, in another the cells were green with only traces of haematochrome. When first gathered it was almost impossible to distinguish any protoplasmic strands even with staining, and at first it was doubted whether the alga was in fact *Haematococcus*, but by the next afternoon the strands were becoming more distinct. In the morning when first collected, many small non-motile cells were present in various stages of division. Between 5 and 7 p.m., in the orange-coloured material there were many small rather rounded cells, red in colour, moving actively among the larger cells with a characteristically steady motion. Although the

membrane had not noticeably separated from the protoplast there was obviously a wall present and later as the cells developed it became quite clear that these were indeed typical young motile cells of *H. lacustris*.

In the green material, on the other hand, mixed with the typical *Haematococcus* motile cells in which the protoplasmic strands were now faintly visible, were very many extremely active naked motile cells, much smaller and elongated in shape. Some were conjugating, while others, quadriflagellate, were newly formed plano-zygotes, showing two eyespots (fig. 6, N). In this material no definite pyrenoid could be distinguished in the swarmers but abundant stroma starch was present. They were slightly larger than the gametes of *H. capensis* and less elongated, but otherwise not noticeably different.

Recently, early in 1958, my attention was drawn to Peebles's paper and as two strains of *Haematococcus lacustris* were available at the time—one brought from Rooi Krantz on the upper Swartkops River by A. R. A. Noel, the other collected in several tiny rock-pools at Signal Hill above Grahamstown—attempts were made to apply her methods to obtain gametes. The two strains differed considerably in behaviour even when treated similarly. Juller's culture solution was added to water in which the alga was growing and with this treatment the Signal Hill material produced growths which were almost pure green whereas the Rooi Krantz material continued orange-red in colour and showed a strong tendency to pass into the non-motile state.

Material from each was put into small pyrex evaporating dishes lightly covered with watch glasses. Rich growths resulted and as the water-level slowly dropped, a rim of green or slightly reddish quiescent cells formed at the edge of the water; since the cells forming it were in a moist atmosphere they never dried out completely and even in the Rooi Krantz cultures never became completely red. When the rim of cells had reached a fair size, the liquid was poured away and the dishes inverted into saucers after rinsing with glass distilled water. Next morning sufficient 50 per cent culture solution was poured into the dishes to just cover the rim of cells. After a few hours in nearly every case many naked swarmers were present—more in the case of the Signal Hill than in the Rooi Krantz cultures.

In some of the Signal Hill cultures the swarmers were small ($2{\cdot}5 \times 4{\cdot}5\mu$) and, though no conjugation was observed, a few rounded cells with two eyespots (presumably zygotes) were seen. In these cultures the swarmers were more or less fusiform with a small but distinct apical papilla and posterior pole either rounded or pointed, with a well-marked pyrenoid and eyespot (fig. 6, O), and were pale green in colour. The zygotes varied from 5 to 9μ in diameter.

In most of the cultures, however, including all those of Rooi Krantz material in which swarmers were formed, they were considerably larger ($4{\cdot}5 - 7{\cdot}0\mu \times 7 - 9{\cdot}0\mu$), occasionally very much elongated ($3{\cdot}6 \times 12{\cdot}6\mu$) with flagella $11–13\mu$, and in none of these cultures was any sign of conjugation seen. Swarmers from Signal Hill and Rooi Krantz were mixed but still no sign of copulation was found.

The Signal Hill swarmers were pale green without any trace of haematochrome while those from Rooi Krantz usually had some haematochrome or, if green to begin

with, after a couple of hours on agar they began to show traces of haematochrome. The appearance of the red pigment was very pretty—the anterior apex in the gametes was very well differentiated and after a time began to show a slight tinge of red (cf. Hazen, l.c., pl. 86, fig. 28). Material from the separate cultures and also a mixture of the two was put on agar plates so that it was easy to watch their development. The drops always contained large motile cells as well as swarmers and on agar the motile cells almost immediately came to rest and rounded off while the swarmers usually collected in a rim round the large cells, kept away from its protoplast by the expanded wall (fig. 6, Q). Where the swarmers were numerous they also settled down in small groups but where possible seemed to prefer the neighbourhood of the larger cells. Where drops of Signal Hill and Rooi Krantz material had been mixed on the agar, it was quite clear which swarmers had come from Signal Hill and which from Rooi Krantz. In the latter, as they rounded off the 'blush' passed from the clear area on one side to the centre of the cell where haematochrome granules soon appeared. In the swarmer the nucleus lies in the clear apex; evidently the formation of haematochrome begins in the nucleated area and when the nucleus assumes the central position, the pigmented zone moves with it. On agar, both types of cells secreted walls and entered on a period of growth. Those from the vegetative cells enlarged very much; those formed by the swarmers also enlarged but to a much smaller degree. Even after enlargement had progressed considerably it was still possible to determine the source of individual 'resting' cells—resting only in the sense that they were not motile; they were actually synthesizing actively as they grew in size.

After some weeks both types of cells were removed from the agar and transferred to 50 per cent culture solution, where both began to germinate, producing numbers of zooids which quickly developed into normal motile cells. The smaller cells naturally produced fewer daughter cells than the much larger resting cells formed by the vegetative motile individuals, but apparently the process of germination and the type of daughter cell produced were the same in both, differing only in the number of daughters produced by smaller cells.

These experiments are not conclusive and far more research is needed on the whole subject of the naked swarmers produced by this species. So far as they go, they seem to indicate that two types of swarmers are produced, the smaller being true gametes which must in all probability undergo conjugation before any further development can take place—and the second type, considerably larger although naked, being of the nature of accessory cells which do not conjugate but form resting spores. These latter, like the cells formed by the ordinary vegetative cells, ultimately germinate to produce young motile cells. If this is correct, it would explain much of the discrepancy as to size and behaviour of naked swarmers which is reported in the literature.

Obviously, cytological investigation is urgently needed at all stages of development, but especially of the different types of resting cells at germination. As yet, there are no records of the development of zygospores and their behaviour on germination.

DISCUSSION

I. Cell Structure

Throughout the genus the dominant form is the motile cell. Workers who are familiar with the conspicuous and often very large red non-motile cells of *Haematococcus lacustris* are apt to overlook the fact that they represent a *secondary* phase, which can only come into existence after a period of active growth and multiplication of the motile cells. When conditions are normally favourable, e.g. in temporary rain-water pools with a small proportion of mineral salts and possibly at least traces of organic matter, and in cultures which approximate to the natural conditions, *H. lacustris*, just like the other species, produces a rich growth of motile unicells. It differs from all the other species as yet described, in that these cells, although they may be predominantly green, yet nearly always show some development of red pigment which under certain conditions may become the dominant characteristic. To obtain a true picture of its structure and behaviour it is absolutely essential that it be studied in its natural conditions. Growth in culture media of varying composition is invaluable to determine the effect of external conditions on its behaviour, but it cannot be too strongly emphasized that such cultural experiments must be regarded as supplementary to study in natural conditions.

In all the species the basic construction of the cell is the same, but certain details of structure vary in the different species and provide the basis for specific diagnosis. To summarize, the features which all have in common and which together distinguish the genus *Haematococcus* from all other unicellular genera are:

(*a*) The characteristic wall, comprising a firm outer membrane inside which is a clear zone of varying width; filled with a very dilute, watery, slightly mucilaginous liquid.
(*b*) A protoplast considerably smaller than and differing in shape from the outer membrane and attached to the anterior pole of the latter by the flagellary apparatus.
(*c*) A suspensory mechanism consisting of protoplasmic processes traversing the mucilaginous inner region of the wall and connecting the protoplast with the outer membrane.

Of these three features the first needs little further elaboration here, but the remaining two need further discussion since it is largely on the variations found in these characters that specific differences are founded.

(*a*) *The wall*

The wide space between membrane and protoplast is regarded as a part of the wall, but its contents are very dilute, staining only faintly with such a vital stain as methylene blue.

(*b*) *The protoplast*

The protoplast is most often more or less pear-shaped but varies considerably both in shape and size with external conditions, nutrition, temperature, etc., and also with the age of the cell. At times it may ke extensively vacuolated, vacuoles of different sizes lying near the surface of the protoplast and containing granules and globules of various sizes; in other cases such vacuoles are few and with improved conditions they may diminish in size and number, e.g. in the Bonteberg culture (figs. 1, C–E).

Most characteristic is the flagellary apparatus with its two curious structures, the 'flagellary tubes' through which the flagella traverse the inner zone of the wall before emerging on opposite sides of the apex. The length of these tubes depends mainly on the distance of the apex of the protoplast from the membrane and partly on the angle of divergence between them. In *H. buetschlii* according to Blochmann the apex actually reaches the membrane and the short flagellary tubes diverge at an angle of 180°, the flagella emerging on opposite sides of a very flat apical papilla. In *H. droebakensis* and *H. capensis* although the apex of the protoplast is very close to the membrane, there is yet an appreciable distance between apex and membrane; the flagellary tubes are short and the angle between them is somewhat variable but is usually less than a right angle; where there is an apical papilla the flagella emerge on opposite sides of it. In *H. zimbabwiensis* the distance between apex and membrane is considerably greater, almost as great as in *H. lacustris*, and the flagellary tubes diverge at a widely obtuse angle, emerging on opposite sides of a very wide, flattened bilobed apical papilla (fig. 5, J). Finally, in *H. lacustris* the protoplast lies approximately centrally within the membrane, the space between the latter and the apex is wide, the flagellary tubes diverge at a right angle and they are longer and more obvious than in any of the other four species (Pl. I, A; figs. 6, A, B, D–H, etc.).

The presence or absence of the apical papillae and their size when present seem to be subject to considerable variation. Where present the papilla is always bilobed and may be conspicuous. But at other times, even in the same species no papillae may be distinguishable, the anterior pole of the membrane showing a smooth curve (cf. the figures of various forms of *H. capensis*). Hence considerable caution is needed in using the form of the apical papilla as a specific character. In *H. lacustris* although the young daughter cell possesses a most conspicuous dome-shaped apical papilla, no such structure is present on the wall of the adult cell. The papilla here is actually an apical protrusion of the protoplast from which the colourless anterior pole develops within the membrane, the flagellary tubes arising from its apex.

The question of contractile vacuoles is a vexed one on which very contradictory statements have been made. Many workers deny that they are present in *Haematococcus*; others, for instance Wollenweber, describe them in detail. Since contractile vacuoles appear to be an integral part of the flagellary mechanism of the motile cell of Chlorophyceae in general, one or more probably occur near the base of the flagella in all species, as Blochmann records for *H. buetschlii* and *H. lacustris*, but if so, they are very inconspicuous and difficult to distinguish, in this respect differing markedly from most species of *Chlamydomonas*, for instance (see fig. 1, D, F; fig. 6, B). The numerous contractile vacuoles scattered over the surface of the protoplast in *H. lacustris*, recorded by various workers including Wollenweber, are rather a different matter, not directly concerned with the flagellary mechanism. Wollenweber (1908, p. 274) states that he was able to observe them in both artificial culture and under natural conditions, scattered over the whole surface of the protoplast to a number of between 30 and 40, 'possibly at the spots where the protoplasmic strands radiate out'. I have never been able to observe them in healthy cells but occasionally when a cell has been kept under the cover slip for a prolonged period, in little water, contractile

vacuoles have appeared on the surface, comparable to those found in Volvox spp. (§ globator) near the basal protoplasmic strands (cf. Pocock, fig. 1, A–D).

The chloroplast shows considerable variation, its form being closely connected with the number and distribution of the pyrenoids. In *H. lacustris* it forms a fairly massive parietal sheet, lining the whole cell, with the exception of a small apical portion which is colourless, and enclosing a varying number of pyrenoids scattered more or less evenly throughout its extent. In *H. buetschlii*, *H. droebakensis* and *H. zimbabwiensis* the chloroplast consists of two massive polar portions, each containing a single large pyrenoid, connected by a thin pigmented girdle lining the wall and enclosing the large conspicuous central region, in which the nucleus lies eccentrically, sometimes near the anterior, at other times nearer the posterior pole (fig. 5, A–F, J). Later in the development of the cell, usually marking preparations for division, one or more secondary pyrenoids may appear in each polar portion but always the arrangement in two groups persists. Finally, in *H. capensis* the chloroplast is bowl-shaped with a massive basal portion in which the large pyrenoid lies and extending from which a thinner green zone encloses the central vacuole, much less conspicuous than in the two-pyrenoid species. Towards the apex the walls of the bowl become gradually thinner until they finally disappear leaving a comparatively extensive colourless portion extending to the anterior pole. The nucleus lies immediately above the pyrenoid, nearly in the centre of the cell (fig. 1, A, B and E). Here too secondary pyrenoids may appear as preparations for division begin.

The structure of the protoplast in *Haematococcus capensis* thus approaches most nearly to that of the well-known 'typical' *Chlamydomonas* cell; other species of *Chlamydomonas* however have chloroplasts that approach the second type, with two polar portions connected by an equatorial girdle.

The eyespot is usually conspicuous, somewhat elongated, and in the motile cell more or less equatorial in position, lying on or just within the surface of the chloroplast. In *H. lacustris* however it is often small and inconspicuous, even when not obscured by haematochrome. There is considerable variation in different strains of this species, but on the whole it is less markedly phototactic than any of the others. Perhaps the most interesting feature of the stigma is the way in which it changes its position in the cell, chiefly in connection with cell division, and after this is complete, with maturation of the daughter cells. Since this has been described in some detail in the case of *H. capensis* it is not necessary to describe it again here. Movements of the stigma and of the nucleus at such times serve to emphasize the extreme plasticity of the protoplast and its parts, nowhere more clearly seen than in this genus.

(*c*) *The protoplasmic processes*

The protoplasmic processes are an even more characteristic feature of the genus than the separate wall-membrane, since the latter is known in other genera, for instance *Sphaerellopsis*, some species of *Chlamydomonas*, etc. The combined effect of these processes is to form a very efficient suspensory mechanism, supplementing the flagellary apparatus in keeping the protoplast in position within the membrane. At first sight it may appear that there are two very different types of process but a closer

study of their nature and the existing evidence as to their probable origin serves to a considerable extent to correct this impression. Much of the confusion is directly due to faulty or insufficiently careful examination of the living cell. In all species, the distal portion, that is the part of the protoplasmic process which is connected with the outer membrane, is a fine, thread-like, colourless structure, often but by no means always branched. In *Haematococcus lacustris* these filaments are very numerous and reach from the membrane to the surface of the protoplast. In young growths they may be so fine that they are only discernible with difficulty even when stained. At other times they are thicker, sometimes with granular and vacuolar inclusions; this usually occurs in older growths, and occasionally in such cases, at the points where the filaments are attached to the protoplast the latter may be drawn out into small protuberances as described above (p. 20, fig. 6, B–D). In the other species such protoplasmic protuberances are fewer but much larger and better developed. They never extend to the outer membrane but are connected to it by finer colourless filaments.

II. Asexual Reproduction

The process of asexual reproduction is basically the same in all the species—normally the cell remains motile throughout the whole process until the escape of the anterior daughter cell finally throws the flagellary apparatus out of action. Preparation for division first becomes apparent by the changing shape of the protoplast and the rearrangement of its parts. The most striking feature is the elongation of the anterior pole to form a long beak-like structure, combined with a massing of the main part of the protoplast in the posterior part of the cell. These changes are usually accompanied by withdrawal of the protoplasmic processes particularly those in the anterior part of the cell. The chief variation lies in the angle of inclination of the first plane of division to the polar axis of the cell, and though this may to some extent be correlated with the species, it is by no means constant for any one form as becomes apparent when the figures for each are compared, as for instance in *H. capensis* var. *piriformis* (fig. 4, E–H). On the whole, in *H. droebakensis* it is greater than in *H. capensis* so that the first division may be almost transverse to the cell. Apparently this is even more marked in *H. buetschlii*; Blochmann describes the plane of first division as 'somewhat oblique' to the longitudinal axis of the cell; some of his figures show it as almost transverse to the axis (T.I., fig. 6, 13 T.II 18), but in others, e.g. T.II 20, it was apparently very slightly inclined to the polar axis. In *H. lacustris* it is very variable—in some cells it is nearly parallel to the polar axis (fig. 6, D), in others more or less steeply inclined to it. But whatever the relation of the plane of first division of the protoplast to the polar axis of the cell, by virtue of the rearrangement of the organelles of the protoplast, the first division is essentially longtiudinal, with respect to the protoplast, as are all succeeding divisions.

In the second division *H. lacustris* again differs somewhat in its behaviour, showing a marked tendency for the first two daughter cells to change their relation to one another, separating slightly before the second division takes place. As pointed out above, in this species it is particularly easy to follow the changing position of the

nucleus since the sheath of haematochrome which usually more or less surrounds it moves with it.

The 'double headed' stage in the anterior daughter cell, first mentioned and figured in *H. buetschlii* by Blochmann (tab. 4, figs. 8 and 9) and recorded by Wollenweber in *H. droebakensis* and by both workers in *H. lacustris*, is no doubt the normal procedure in all species. In *H. lacustris* again there is some variation in the position of the flagellary apparatus of the zooids; in some cases the apex of the daughter is developed not at its distal end but towards one side (fig. 6, H, J). These differences all serve to emphasize the extreme plasticity of the species.

Separation of the outer membrane from the protoplast apparently always begins at the posterior pole, where it has been observed in both *H. capensis* and *H. lacustris*, thence spreading towards the anterior end. The exact mechanism has not been clarified, but presumably as separation takes place protoplasmic strands are left uniting protoplast and membrane; apparently the actual separation is brought about by a contraction of the protoplast, probably due in part at least to loss of water, and an expansion of the membrane resulting from increased osmotic pressure within it and hence directly controlled by the protoplast. Further examination of developing daughter cells with the oil immersion lens should help considerably in elucidating the problems of the wall and its formation. Once the protoplasmic connections between protoplast and membrane have disappeared, they cannot be reformed.

In *H. droebakensis* Wollenweber (1908, p. 261) records that motile cells may encyst to form 'aplanospores' of a 'red-ochre colour'; in *H. buetschlii*, however, Blochmann failed to find any such aplanospores either in nature or in his cultures and believed that the sexually formed zygospores constituted the only form of resting cell. In *H. capensis* occasionally similarly formed resting cells have been seen (cf. Pl. II, B), becoming reddish in colour, but as in the case of the zygospores, of a different shade of red from that characteristic of *H. lacustris*.

It seems probable that the power to form such aplanospores at will by encystment of the motile cells exists in all the species, but only in *H. lacustris* has it developed to such a degree that as a result the whole life history has been modified. It seems indubitable that the success of this cosmopolitan species is intimately connected with the very efficient mode of formation of resting spores which enables it to survive and multiply independently of a sexual process.

III. Sexual Reproduction

Sexual reproduction in *Haematococcus* was first described by Blochmann (1886, pp. 450–8) in *H. buetschlii*. Blochmann's up-to-date methods, using the hanging-drop technique and advocating the use of pure cultures, are remarkable and his figures admirably clear. Judging from his description the process is essentially the same as in *H. droebakensis* (Wollenweber, 1908, p. 260) and in *H. capensis*, differing only in a few minor details of which the most significant are (1) the divergence of the plane of first division from the longitudinal axis of the cell. He describes this in the case of asexual reproduction but makes no further mention of this point while describing the division of the sexual cell. His figures of the latter show the plane of first division as

almost but not quite transverse to the cell. This would result in the pore of the globoid of gametes being more nearly lateral than is usual in *H. capensis*, as is shown in his figure 18, but since in figure 20 it is shown as nearly apical, it is probable that in this species too, as in *H. droebakensis* and *H. capensis*, this feature is very variable. But on the whole, as might be expected from the structure of the cell, the plane of first division diverges more widely from the longitudinal axis of the cell in both those species than is usual in *H. capensis*. It is, however, subject to very great variation. (2) The number of gametes (32 or 64) given by Blochmann in *H. buetschlii* is smaller than is usual in either *H. droebakensis* ('over 100' according to Wollenweber) or *H. capensis* where 256 is usual and 512 often occurs. On the other hand, in small cells of the latter species, 32, 16 and on rare occasions as few as 8 may occur. Obviously this too is a variable quantity. What neither of these workers shows is the orientation of the cells in the globoid, the nucleated apices directed inwards so that as the flagella are formed they too are inside the globoid. In short, the similarity to the development of the germ colony of *Volvox* is close (fig. 3, O and Q).

In the case of *H. lacustris* however a very different state of affairs exists. Probably as a result of the perfecting of the efficient system of non-sexual reproduction, the formation of gametes by division of the motile cell has entirely disappeared from the life cycle. The asexually formed hypnospores are naturally formed much more rapidly than the sexually produced zygospores and are in most ways just as effective, indeed serve to multiply the alga to an extent impossible in the latter. As already pointed out, analagous cases of the more or less total replacement of sexually formed reproductive bodies by those formed asexually (or vegetatively) are well known in most groups of plants. Among the Chlorophyceae one of the best-known cases is that of *Hydrodictyon reticulatum* where the asexually produced daughter net has largely replaced sexual reproduction, although the other two species, *H. africanum* and *H. patenaeforme*, still depend mainly on the latter.

There is still considerable uncertainty as to the formation of the gametes in *H. lacustris* and their behaviour. So far as the first is concerned, the generally accepted view now is that they are formed only on germination of young apogamously formed resting cells as described by Peebles (1909, p. 516). Droop (1956A, p. 62) states that in nature they are formed 'only in the 24 hours after rain'. Apparently he did not himself find them in nature but obtained them in culture; he gives no information however as to how they were obtained.

Most of the earlier workers—Flotow (1844), Braun (1851), who seems to have been the first to describe the 'microzooids' accurately, Cohn (1851), Hazen (1899) among them—and later Wollenweber (1908) and Reichenow (1909), to mention only a few who have written on this subject, observed naked swarmers which they suspected to be of the nature of gametes although none of them observed conjugation. It was left for Florence Peebles (1909) to solve the nature of these cells by watching conjugation between them. But neither she nor Schulze (1927, p. 539), who repeated her work, were able to watch the subsequent development and germination of the zygospores. Schulze, incidentally, gives a very full and complete bibliography. Elliott (1934) failed to obtain conjugation. Some workers state that the swarmers can develop

parthenogenetically, others deny this. Further, there is considerable discrepancy in the record of the size of the swarmers.

The results obtained in the course of the present research have already been described The following facts are the most pertinent:

(1) Gametes have been obtained at least once in natural pools the day after rain; they were small, only slightly larger than those of *H. capensis*; conjugation was actively in progress and numbers of quadriflagellate planozygotes were present. Unfortunately their further development was not followed.

(2) During the past year (1958) Peebles's method of obtaining gametes was applied to three strains from different localities: Signal Hill near Grahamstown, Rooi Krantz in the Uitenhage mountains, and Buffels Bay. Swarmers were obtained from all. On one occasion from the Signal Hill material the swarmers were small and though conjugation was not seen, a few zygotes showing two eyespots were observed (fig. 6, O).

(3) In most cases the swarmers obtained from both sources were considerably larger (fig. 6, P); no signs of conjugation were seen and when put on agar plates, the swarmers, like the vegetative motile cells, settled down, secreted a wall and underwent a period of growth. Despite considerable enlargement, they remained smaller than the resting cells formed from the motile cells (fig. 6, Q), but like them, eventually germinated similarly (when treated appropriately), producing typical motile cells, though fewer in number.

These results are admittedly incomplete and much further work is needed. Such as they are, however, they suggest that two types of naked swarmers may be formed from resting cells. (1) The smaller, very slightly larger than the gametes of *H. capensis* and not unlike them, are true gametes which conjugate to form zygotes. Should they fail to conjugate, the probability is that they would soon disintegrate. (2) The second type of swarmer is larger and is of the nature of accessory cells. These do not conjugate, but settle down, secrete a wall and form resting cells which ultimately germinate to form typical motile cells.

If these results are confirmed, they would go far to explain the discrepancies in the various accounts, particularly the variations recorded as to size and form of the 'gametes' and the possibility of 'parthenogenetic' development.

Obviously, in addition to further cultural work and observations of natural growths, what is urgently needed is a full cytogenetic study. As yet we know little as to the nuclear equipment of *H. lacustris*; Elliott's cytological study (1934, p. 258 etc.) deals only with the vegetative cell and its division, and at what stage meiosis takes place is still unknown.

IV. Taxonomic Position

Droop (1956 B), the most recent writer on *Haematococcus*, argues for the rejection of the name *Haematococcus lacustris* (Girod-Chantrans) Rostafinski in favour of the combination *H. pluvialis* Flotow.

In a report of observations by Girod-Chantrans (1797) '*correspondent* (*de la Société Philomathique*) *à Besançon*' it is stated that he had observed a 'volvox' not described by Linnaeus which showed some resemblance to '*le globator*' (Gmelin) but differed from it considerably; this he identified with an organism recorded by Haller. Five years later (1802), under the heading 'No. 17 Conferva No. 2109 (Haller) hist. pl. helv.', Girod-Chantrans gave an account of the occurrence and behaviour of an organism which had appeared 'spontaneously' in a vessel in which he was trying to '*revivifier la Conferve glomerata dessechée depuis plusieurs mois . . . par le simple procédé de l'immersion*'. To his great surprise the water turned red and he found that it contained a 'volvox' which swam with a movement caused by a rotation on its axis combined with a forward progression, while the surface of the conferva and later the sides and bottom of the vessel containing it were coloured red with rounded resting cells. This he identified with Haller's specimen No. 2109 and with the material he had observed at Besançon, giving it the name of '*volvox lacustris*'.

Later (p. 186), '*addition au No. 17 ci-dessus*', he amplified his previous description with further observations on the behaviour of the organism. Nowhere is there any suggestion that the alga originated from salt or brackish water as suggested by Cohn, who acting on this supposition decided that Girod-Chantrans was not dealing with *Haematococcus* and consequently rejected his name for the alga.

An interesting detail emerges from a study of the figures given by Girod-Chantrans; his figures 17′—17‴ are of *H. lacustris*, figure 17′ shows the motile stage, figure 17″ rounded cells at rest on some surface, figure 17⁗ the same covering the surface of the 'conferva', but figure 17 which he includes to show the range in size appears to be a low-power drawing of a volvox-colony with ripe oospores. It thus seems possible that his original material may have included some *Volvox*. However, from the remaining figures and from his description, based on the organism which he had obtained in his culture and its behaviour, it is quite clear that his *Volvox lacustris* and *Haematococcus pluvialis* Flotow are one and the same organism.

With this conclusion Droop entirely agrees but concludes with the curious statement (p. 184): 'Even if there is reasonable certainty that *Volvox lacustris* and *H. pluvialis* are synonyms, the diagnosis is scarcely good enough to warrant the adoption of *lacustris* as the specific name.'

According to the International Rules of Botanical Nomenclature a worker is not entitled to reject an earlier name merely because he considers the original diagnosis as 'scarcely good enough'. However strongly we may feel that *pluvialis* is a more appropriate specific epithet for this alga, Girod-Chantrans's name *lacustris*, as Rostafinski pointed out, is undoubtedly valid and has priority. The name *Haematococcus lacustris* (Girod) Rostafinski must therefore stand. Should it later be proved necessary, the generic name *Haematococcus* should be proposed for conservation.

Droop (1956 A, p. 63 et seq.) further proposes that the existing genus *Haematococcus* should be divided into two genera, the original name being retained for the species *H. lacustris* (he uses the combination *H. pluvialis* but for the sake of clarity I use *H. lacustris* throughout), while for the two species *H. buetschlii* Blochmann and *H. droebakensis* Wollenweber, to which the two new species, *H. capensis* and *H. zim-*

babwiensis, described here, must obviously be added, he proposes to create a new genus *Balticola*, the diagnosis for which 'is based mainly on five or six strains in culture at Cambridge' (p. 64).

Here I fear that I must take strong exception to Droop's conclusions which seem to me to be based largely on insufficient and at times inaccurate observations. Further, he has obviously neglected the study of natural growths of the algae in question, particularly in the case of *H. lacustris*, depending too much on laboratory cultures, thus again showing ignorance of the Rules of Nomenclature.

The grounds given for the separation of *H. lacustris* from the other two species are the following:

(*a*) Pyrenoid number (p. 56). True, but this cannot be regarded as of more than specific significance.

(*b*) Pyrenoids in *H. lacustris* usually undergo fragmentation before the onset of cell division, resulting in a pyrenoid-free protoplast during division. Even if true, this again can hardly be taken as a generic character, but it is not even invariably the case. Sometimes it is true that in this, as in the other species, the pyrenoids disappear in the early stage of cell division, but dividing material of *H. lacustris* treated with aceto-carmine often shows the presence of pyrenoids in the cells even at a late stage of division and the same varying behaviour of the pyrenoids is true also for *H. capensis* and *H. droebakensis*.

(*c*) 'In *H. lacustris* the plane of first division is longitudinal' (p. 56). *H. droebakensis* has 'a truly transverse first division' (p. 63) . . . 'typical early stages of division show that the dividing plane could not have come to lie transversely through rotation of the protoplast owing to the latter's length and the continued attachment of the flagella' (p. 57). These statements show not only a lack of accurate observation, but also a complete misunderstanding of the occurrence and significance of the rearrangement of the parts of the protoplast preparatory to the division of the cell in the Volvocaceae (see Fritsch, 1935, p. 93; Pocock, 1933, pp. 587, 620, etc.). In any vigorous growth of *H. droebakensis* in which division is in progress a series showing the process of rearrangement within the protoplast is easily found.

(*d*) 'Prior to division the flagella of *H. lacustris* become detached from the protoplast' (p. 57). Yet nearly all the many workers who have written about this alga, many of them outstanding for the care and accuracy of their observations, have described and figured division as taking place in *motile* cells! One can only suppose that, as seems probable from the composition Droop gives for the culture medium he was using, he was dealing with somewhat abnormal cultures, entirely neglected to observe natural growths and ignored the accounts given by previous workers. Dividing cells in early stages of division or with two or four daughters present within the parent cell, always motile, are so striking a feature of any vigorously dividing growth of the alga when growing under normal conditions that it is difficult to understand how anyone studying it could overlook them. Yet on p. 59 Droop repeats his previous statement as to loss of motility in dividing cells of *H. lacustris* making the very curious observation: 'on the other hand *H. pluvialis* loses the power of movement *soon after the flagella*

have become detached from the protoplast at their base' (italics mine) and adds: 'indeed the flagella in this species are normally thrown off completely before the end of the first division. Instances of motility during the later stages of the second division are due to *puncturing of the parent membrane by the daughter flagella*' (italics again mine). How does Droop imagine such a process could take place?

Later (p. 63) Droop sums up the characters which he considers distinguish *H. droebakensis* from *H. pluvialis*, including that in *H. droebakensis* the cells are distinguished by:

(*a*) Possessing a papilla.
(*b*) Possessing thick branched protoplasmic strands which may contain portions of the chromatophore.
(*c*) Having mostly two pyrenoids.
(*d*) Pyrenoids tending to be distributed whole among the daughter cells.
(*e*) Having truly transverse first division.
(*f*) Retention of motility throughout the process of cell division.
(*g*) Retention of parental flagella and stigma by one of the daughter cells of each division.
(*h*) Having no asexual resting spore (but cf. Wollenweber, 1908, p. 261).
(*i*) Not accumulating haematochrome in the vegetative cells.
(*j*) Having gametogenesis in motile cells.
(*k*) Having fusiform gametes tapering posteriorly, which are possibly not facultative.

On these grounds he proposes to create a new genus, *Balticola*. Examining the characters thus enumerated we find that:

(1) An apical papilla, even when it occurs in a species of *Haematococcus*, has been shown in this investigation to be a very variable character; hence its presence or absence, while sometimes useful as a specific distinction is scarcely stable enough to be regarded as of generic significance.

(2) As regards the protoplasmic processes, Droop entirely ignores the fine thread-like distal portions which connect the thick, chlorophyll-containing processes to the wall of the cell. These are present in the living cell of all species, but since in *H. lacustris* the protoplast does not normally form thick protrusions, the strands connect protoplast and wall directly.

(3) The 'truly transverse division' as pointed out above does not exist: division of the cell is always longitudinal in respect to the protoplast.

(4) Retention of motility during division is common to all species, not excepting *H. lacustris*.

(5) Gametes are so strongly metabolic, altering their shape according to the speed of their movement that the absence of a 'pointed posterior pole' can scarcely be regarded as a generic character, nor is it always true (cf. fig. 6, O, P). Droop's description of the 'gametes' of *H. lacustris* differs from that given by other workers. The 'possibly not facultative' again can hardly be accepted as a generic character.

Hence the only remaining differences are: (1) difference in pyrenoid number, (2) not accumulating haematochrome in the vegetative(? motile) cell, (3) having gametogenesis in the motile cell. Up to a point these are useful for differentiation of species but cannot be accepted as generic characters. Even the two most constant and significant features—one structural (the presence of the stout proximal portions of

the protoplasmic processes), the other developmental (the perfecting of a non-sexual formation of resting cells with the consequent changes in the life history)—cannot be so regarded when weighed against all the characters the algae have in common.

I am therefore unable to accept the genus *Balticola* Droop. As at present known, the genus *Haematococcus* may be divided as in the following key:

KEY TO THE SPECIES OF HAEMATOCOCCUS

A. Protoplasmic processes very many, thread-like and colourless throughout
  B. Pyrenoids many
    C. Apex of protoplast blunt, distant from membrane, flagellary tubes long, at right angles to one another
      1. *H. lacustris* (Girod.) Rostaf.

AA. Protoplasmic processes fewer, thick and green at base, connected with outer membrane by fine branching colourless threads as in A
  B. Pyrenoids two
    C. Apex reaching the membrane
      D. Apex elongated, flagella emerging from beneath a flat saddle-shaped protuberance
        2. *H. buetschlii* Blochmann

    CC. Apex not reaching the membrane
      D. Apex elongated, nearly reaching the membrane, flagellary tubes short, bilobed apical papilla present
        3. *H. droebakensis* Wollenweber

      DD. Apex stumpy, distant from the membrane. Apical papilla bilobed, flat and broad, flagellary tubes long, at an obtuse angle.
        4. *H. zimbabwiensis* Pocock sp. nov.

  BB. Pyrenoid one
    C. Apex not reaching the membrane
      D. Apex elongated, flagellary tubes short; bilobed apical papilla sometimes present
        5. *H. capensis* Pocock sp. nov.

SUMMARY AND CONCLUSION

Part I

1. Ecological aspects determining the occurrence of *Haematococcus* are outlined, with special reference to those peculiar to Southern Africa.

2. The inception of the research and its development are described and data obtained from the original habitats discussed.

3. Two new species are described: *Haematococcus capensis*, with three varieties and one form, and *Haematococcus zimbabwiensis* from Rhodesia.

4. Notes on observations of *Haematococcus droebakensis* collected at Stonehenge, Wiltshire, and grown in culture at the Cape and in Grahamstown, are recorded.

5. The widespread occurrence of *Haematococcus lacustris* in South Africa is indicated.

Part II deals in more detail with the life histories and behaviour of the various species, more particularly *H. capensis* and *H. lacustris*.

6. In *H. capensis*: detailed studies of the structure of the cell, behaviour, reproduction and general life history are described, primarily in the case of the typical var. *capensis*, but with references also to var. *torpedo* and var. *piriformis* with its forms.

7. In *H. lacustris*: (*a*) In nature, gametes, conjugation and zygotes have once been obtained in pools on rock in the bed of the Palmiet River, near Grahamstown.

(*b*) In cultures, by applying Peebles's methods naked swarmers were obtained in three strains from widely separated localities. These swarmers were observed to be of two sizes: the smaller conjugated to form zygotes and are obviously gametes. The larger showed no signs of conjugation, no zygotes were seen and on agar they settled down, rounded off, secreted a wall and underwent a period of growth after which they germinated to form a number of typical motile cells.

(*c*) The larger swarmers are regarded as of the nature of accessory cells and the suggestion made that previous records of parthenogenetic development of gametes refer to them.

8. Droop's work is criticized and the conclusions reached that (1) the specific name name *lacustris* founded on Girod-Chantrans's species *Volvox lacustris* is valid, has priority over the specific epithet *pluvialis* Flotow, as long since pointed out by Rostafinski, and must therefore be accepted, and (2) that his proposed division of the genus *Haematococcus* into two genera is not founded on reliable data. Hence the genus *Balticola* Droop is rejected.

9. A key to the existing species of *Haematococcus* is given.

In conclusion I take this opportunity to express once more my most grateful thanks to the Botany Departments of the University of Cape Town and of Rhodes University for the working facilities granted me. I am indebted to my brother, Prof. L. G. Pocock, and to Prof. S. Whitely for the Latin diagnoses, and to Dr. M. S. Cave for the two photographs reproduced as Plate I, figure H, and Plate II, figure C. My thanks are also due to the Council for Scientific and Industrial Research for grants for algal research made to me from time to time, since much of the earlier part of this work was carried on while holding such grants.

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* No attempt is made here to give a full bibliography of *Haematococcus*. Both Schulze and Elliott give extensive lists of the relevant literature.

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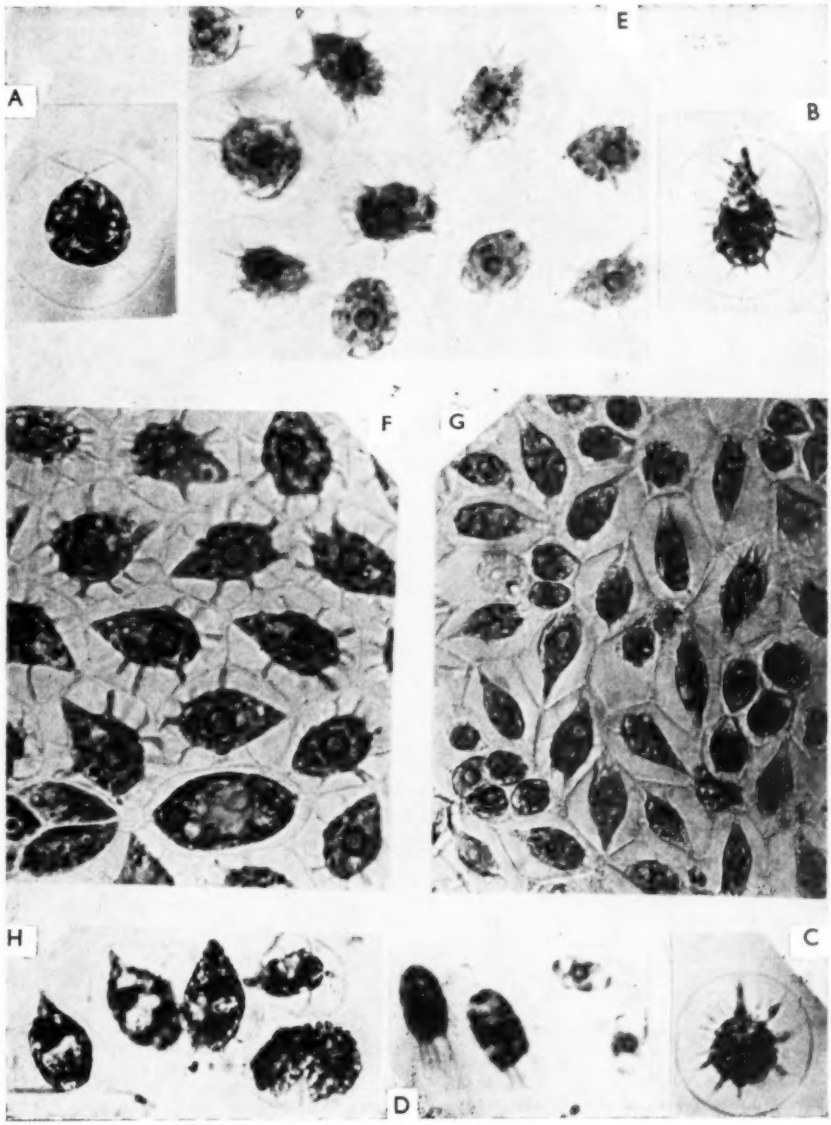
HAEMATOCOCCUS

A–D Motile cells in water.
A. *Haematococcus lacustris* ×390. B, C. *H. capensis* var. *capensis*, De Klip. 2 cells. ×420. D, *H. capensis* var. *torpedo*, Buffels Bay. 2 mature cells showing development of posterior processes. ×520.

E–F *H. capensis* var. *capensis*, Bonteberg. Living, unstained.
E, On agar. ×520. F, Liquid culture with 1 cell of *H. droebakensis*, living. Photo. M. S. Cave. (approx.) ×640.

G *H. capensis* var. *torpedo*, Buffels Bay, culture on agar. Living.
Some cells showing nearly mature zooids ready to escape. ×420.

H *H. droebakensis*, Stonehenge culture (fixed).
5 motile cells, one preparing to divide, and one with nearly mature gametes. ×420.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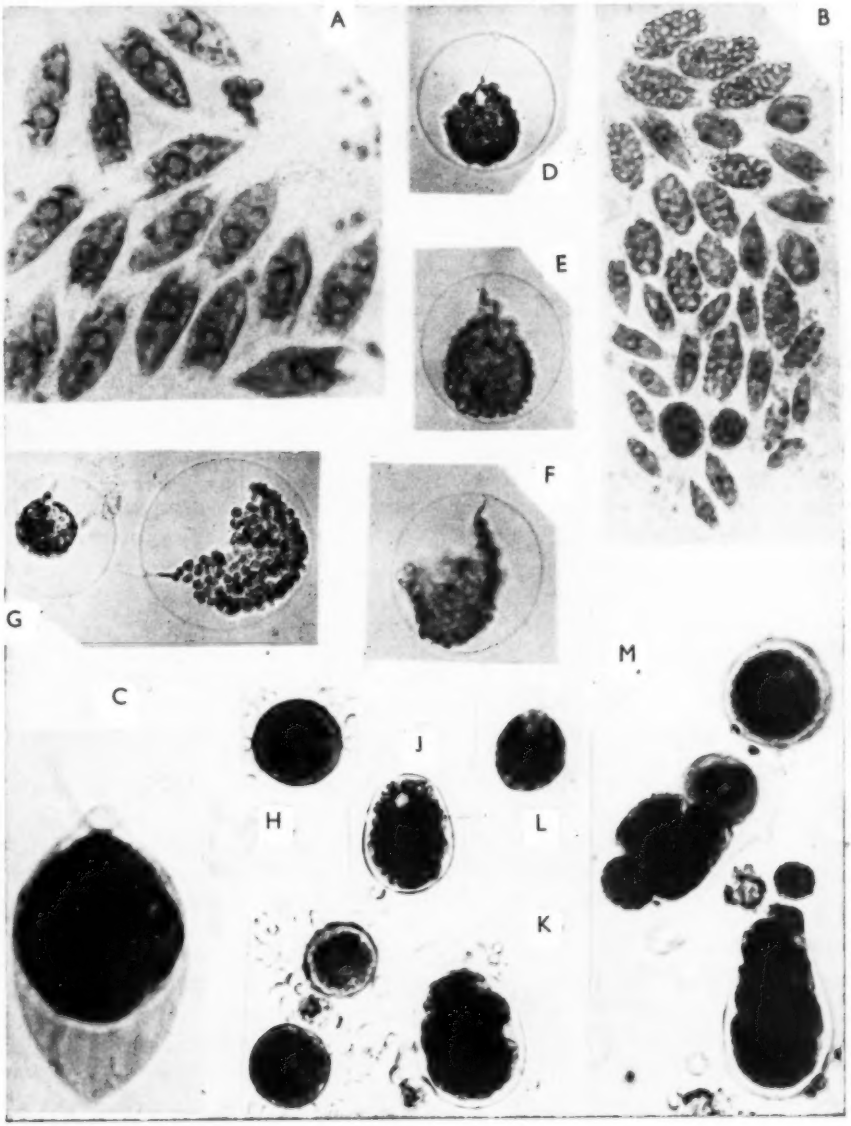
HAEMATOCOCCUS

A–B *Haematococcus capensis* var. *torpedo*, single-cell innoculations on agar.
A. 16 vegetative cells the product of 4 successive divisions. ×640. B. Older group. Three cells rounded off to form resting cells; a number of vegetative cells; the rest in various stages of sexual reproduction. ×375. On the addition of a drop of liquid to the agar cultures all the cells, with the exception of the rounded cells, would immediately become actively motile.

C *H. capensis* var. *piriformis*, Somerset St., Grahamstown. Eye spot equatorial, on right.
Single cell killed with osmic acid vapour, stained methylene blue. Photo. M. S. Cave. × circ. 1,000.

D–G *H. capensis* var. *capensis*, De Klip, sexual reproduction. ×420.
D. Cell division showing young gamete globoid, apex long-drawn out with stigma at base. E. Division almost complete. F. Globoid opening preparatory to breaking up. G. Another (slightly squashed), dissociation beginning, with a vegetative cell rounding off under pressure of the cover slip.

H–M *H. capensis* var. *torpedo*, Buffels Bay, development and germination of the zygospore. ×520.
H. Zygospore still enlarging. J. Zygospore germinating; protoplast already divided into 4. K. 3 zygospores, 2 young, enlarging, the third germinating, protoplast showing cleavage further advanced. L. Zoospore recently liberated. M. One zygospore preparing to germinate; two others, both very large, already divided. The central one shows cleavage into 4 parts, the larger of which probably would have divided again; the one at the bottom right divided into 4 large parts, the apical part liberating 2 zoospores, the second part beginning to divide again, the 2 posterior quarters not yet showing cleavage.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AN ARCHAEOCYATHID FROM THE NAMA SYSTEM

By S. H. Haughton

(With Plates III—V)

(Read March 18, 1959)

Fossils preserved in a quartzitic sandstone of the Kuibis beds from near Ham River, South West Africa, are considered to represent a double-walled Archaeocyathid. The mode of preservation is unusual for this phylum, and it is suggested that more complete specimens may occur in the calcareous members of these beds or in the succeeding Schwarzkalk. The age of the beds is considered to be Lower Cambrian.

During the course of geological mapping in the Warmbad district of South West Africa in 1927, Dr. H. F. Frommurze and the author picked up two small weathered slabs of Kuibis quartzite showing organic remains. The locality was a small gully cut in the low Nama escarpment on Gründoorn near the Ham River. Reference was made to these fossils in 'The Geology of the Warmbad District' (S.W. Africa Dept. Mines, *Memoir* II, 1936), where it was considered that their nature was 'problematical' and that 'in general appearance they bear some resemblance to the Archaeocyathid *Coscinocyathus*'. Since that date, they have remained without further study, although Haughton and Martin published a somewhat blurred photograph of one of the slabs in their paper on 'The Nama System in South and South-West Africa' which formed a contribution to the Symposium on the Cambrian system organized by the XXth International Geological Congress in Mexico City in 1956.

The publication by the Centre d'Etudes et de Documentation Paléontologiques of a translation in French of a Russian paper on the Archaeocyathi by I. T. Zhuravleva (1954) and the appearance of V. J. Okulitch's article on Archaeocyatha in Part E of the *Treatise on Invertebrate Palaeontology* (1955) revived the author's interest in these early fossils from southern Africa and led to this present account.

The two weathered slabs (both labelled S.W. 136 by the finders) are now housed in the South African Museum, Cape Town. To the Director of that Museum, Dr. A. W. Crompton, I am indebted for plaster casts of the specimens and for a beautiful set of photographs some of which are used to illustrate this present paper.

The fossils are preserved as moulds on the bedding planes of a hard, fairly fine-grained greenish sandstone, a typical member of the Kuibis beds which form the basal part of the Nama system in this area. One of the slabs (here referred to as 'A)' shows a lumpy mass at one end which is interpreted as a small colony and a considerable number of detached isolated individuals some of which occur in cross-section. The other slab ('B') has on the one surface several isolated individuals and fragments. In some cases, the mould is marked off from the remainder of the slab

by a bordering depression; several of the individuals show a rough parallelism to one another, as if they had attained their present position in the matrix by slight current action.

Fundamentally, each individual consists of an elongated tubular cone of circular cross-section; the more complete individuals have a pointed lower end which probably served in life to fix the organism to the floor of the water-body in which it lived. In two cases preserved on slab B, two individuals appear to have been joined at this pointed end. Evidence of the approximately circular cross-section of the body is seen on slab A.

Several of the cups show, as preserved, a number of elongate pustulate ridges. Each cup must have carried 10–12 of these ridges; the pustules are regularly spaced. There are, in addition, indications of the presence of small transverse septal constrictions. The approximately circular ridge seen on slab A can be interpreted as an infilling of the intervallum or space between the internal and external walls of a cup or as a replacement of a single thick exoskeleton. If the former, there is no evidence visible in them of the existence of septa or plates within the intervallum; these septa, however, are not always present in the Archaeocyathids, according to both Zhuravleva and Okulitch.

Comparison of the maximum diameter of the ovoid cross-sections on slab A with the width across the cup fragments suggests that the external features of the cups as preserved may correspond to the appearance of the inner side of the outer wall of the living animal. It might then be that the longitudinal section of the bottom part of the elongate cup in the middle of slab B shows, from the centre outwards, first an infilling of the central cavity, then a thin wall (indicated by a depression), a series of parallel subhorizontal tabella between the inner and outer walls, and externally a thin outer wall (also indicated by a depression). If this interpretation be accepted, then the inner face of the outer wall was longitudinally ridged by broad rounded ridges and the longitudinal channels between the ridges were each perforated by a row of evenly spaced pores. From the ridges vertical radial plates (parieties) may have extended inwards to meet the inner wall.

The specimens are of variable size. The largest is that occurring in the middle of slab B; the length of its cup is approximately 32 mm. and the greatest diameter 5·5 mm.

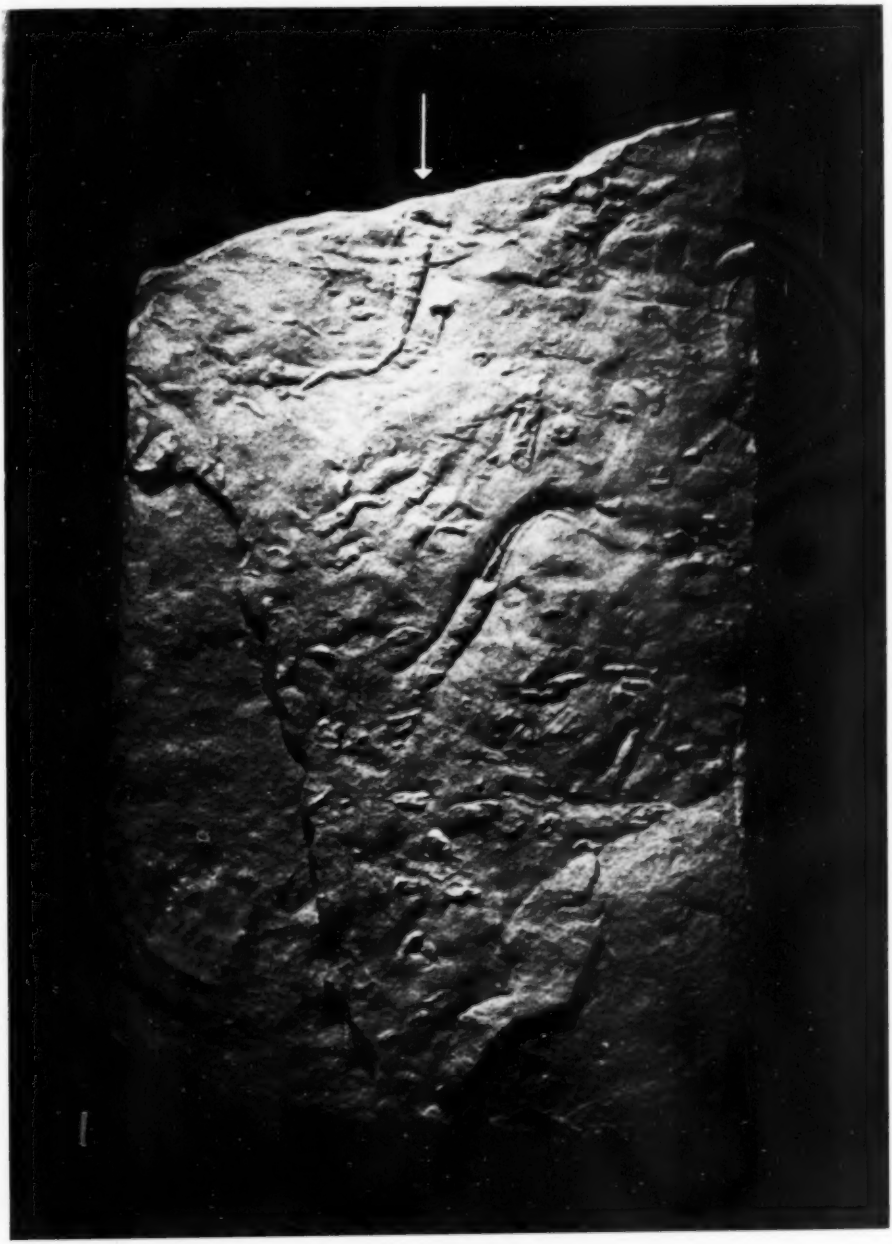
The mode of preservation of these fossils makes any attempt at precise classification hazardous. As sessile forms with a central cavity and apparently with an intervallum carrying tabella, they appear to possess the features necessary for inclusion within the class Archaeocyathea (Okulitch, 1943) of the phylum Archaeocyatha (Vologdin, 1937). Within this class, orders are defined on the basis of wall structure and the nature of the parieties. Essentially, as far as can be seen, the pores in this form are arranged in linear fashion and, although large, occupy less than one-half of the total area of the wall. If the transverse depressions seen on the larger individuals on slab B are correctly determined as the impressions of tabulae, this form may be considered as allied to the family Coscinocyathidae Taylor, 1910. In the absence of details, however, this can only be regarded as a first approximation.

Archaeocyathids have been described from North America, North Africa, Australia and Eurasia; in all these regions they are exclusively Cambrian in age and almost entirely confined to the Lower and the Middle Cambrian beds, with most of the described genera coming from the Lower Cambrian. For the greater part, their remains are found in pure limestones, as if the animals preferred pure and still waters. In Transbaikalia and elsewhere, however, the containing rocks are more or less argillaceous. Zhuravleva has stated that the introduction of a considerable quantity of mud into their environment has usually resulted in certain changes in shape, among which elongation and the adoption of a cylindrical form is known.

The specimens now discussed are unusual in that they occur in a quartzitic sandstone. Nevertheless, in the area of their provenance, the Kuibis beds carry some thin limestone intercalations in the upper members and they are followed conformably by the dominantly calcareous Schwarzkalk series. Detailed search in these calcareous members of the Kuibis and of the succeeding Schwarzkalk ought to reveal further Archaeocyathid material that is sufficiently well preserved to enable detailed studies to be made of their structure by sectioning.

It should be recalled that R. Richter, when describing material from the Kuibis beds earlier considered by Gürich to belong to the genera which he named *Rangea* and *Pteridinium*, considered that these forms should be assigned to the Gorgonaria Holaxonia and that, in consequence, the Kuibis beds cannot be older than Cambrian. He further suggested that they might be younger than Cambrian, even Silurian; but the evidence afforded by the Archaeocyathids is against the acceptance of this suggestion.

Slab B. Slightly above natural size.



Slab A. Approximately natural size.

Slab B. × $\frac{1}{3}$